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Zoology

Statistics

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SYNTHESIS OF Cu-Zn NANO-FERRITE BY OXALATE CO-PRECIPITATION METHOD

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ABSTRACT: *Cu-Zn ferrite with composition Cu0.6Zn0.4Fe2O4 was synthesized by oxalate co-precipitation method. Pre-sintering andsintering of resulting powder was carried out at3000C for 2hr and 600o C for 4 hrs. The ferrite sample characterized by X- ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Field emission scanning electron microscopy (FESEM), Energy dispersive X-rayanalysis (EDAX). The formation of single phase cubic spinel structure of ferrite was confirmed by XRD analysis.The Debye-Scherrer formula was used to calculate the crystallite size of the ferrite. The FTIR spectrum shows two major absorption bands suggested by Waldron. Nano ferrite formation was approved by morphological analysis.EDAX spectra shows the appearance of appropriate elemental composition in the ferrite.*

Keywords: *Cu-Zn ferrite, Co-precipitation method, XRD, FTIR, FESEM*

I. Introduction

Soft ferrites are the important class of magnetic material exhibitsexcellent electrical and magnetic properties as compared to other materials and have several applications in various fields such as electronics, optoelectronic, electrical, automobile, medical, environment etc. The properties of ferrites are sensitive to various parameters such as synthesis technique, sintering temperature, sintering atmosphere [1], sintering time [2],concentration of dopant, impurity doping[3],cation distribution[4,5] etc.

Zinc substituted ferrites have been extensively studied because of its interesting electrical and magnetic properties.Several researchers have been studied properties of Ni-Zn [6-8], Mg-Zn [9, 10], Mn-Zn [11, 12],Co-Zn [13], Li-Zn [14, 15] ferrites.Most of the researchers synthesized Cu-Zn ferrite by ceramic technique [16-18].A little work has been carried out on properties of Cu-Zn ferrites prepared by chemical methods [19-23].Copper ferrite exhibits inverse spinel structure whereas zinc ferrite exhibits normal spinel structure, belonging to the Fd-3m space group and crystallize in face-centred cubic structure. Zinc ferrite material shows an abnormal anti-ferromagnetic behavior havingnearly 10 K Neel temperature(TN).Structural and magnetic properties of spinal ferritesinfluences by Zn2+ substitution.It was found that Zn2+substituted copper ferrite shows improved structural, electrical, optical and magnetic properties [4, 5, 24-31]. On account of cation distribution, Zn2+ ions strongly occupy the tetrahedral site whereas Cu2+ ions occupy to octahedral site while Fe3+ ions can accommodate to both tetrahedral and octahedral sites [32].

Cu-Zn ferrites has wide range of applications because of their fascinating properties and hence they are widely used to fabricatethe electronic components such as transformer cores, radio frequency coil, antennas, and magnetic core of read-write heads for high speed, sensor, switches, isolators [33] etc. These ferrites has excellent soft magnetic properties at high frequencies and high electrical resistivity [20].

Smay et al. [34] have shown that electrical and magnetic properties of Cu-Zn ferrites are influenced by the substitution of Nd3+ ions. Abbas et al. [35] have been prepared Cu-Zn ferrites by solid state reaction method and shows that lattice parameter, X-ray density, grain size, bulk density and porosity were changed with sintering temperature. Effect of zinc substitution on microstructure and magnetic properties of Cu-Zn ferrites were reported by Rana et al. [24]. They observed that the porosity, grain size,Curie temperature and coercivity of Cu-Zn ferrites were decreases withincreasing Zn2+ content.Samy [37] synthesized rare earth substituted Cu-Zn ferrite by ceramic technique. He observed that electrical resistivity, initial permeability and homogeneity increases whereas magnetization and energy loss of ferrites decreases with increasing rare earth content.

In present work, we prepared Cu-Zn ferrite by oxalate co-precipitation technique at lower sintering temperature and studied its structural properties

II. Experimental

Soft ferrite with chemical formula Cu0.6Zn0.4Fe2O4 was synthesized by oxalate co-precipitation method. Thomas Baker make AR grade chemicals such as copper sulfate (CuSO4-5H2O, 99.0%), zinc sulfate (ZnSO4-7H2O, 99.0%), iron sulfate (FeSO4-7H2O, 99.5%) and ammonium oxalate (COONH4)3-H2O, 99.5%) were used for the synthesis of ferrite material. Initially required amount of sulfates were weighed using digital microbalance and dissolve in double distilled water. The process of complete dilution of chemicals was obtained by adding drop wise con. H2SO4. For molecular level mixing,solution was heated until itsvolume reduces to its half.The precipitate of mixed metal oxalates was obtained by adding saturated solution of ammonium oxalates into the cooled solution of sulphates. The precipitated solution was placed on the sand bath for digestion. TheBuckner funnel with 41 size Whatman paper followed by vacuum pump was used to filtrate the precipitated solution.In order to remove sulphate ions, the precipitate was washed several times.The assurance of removal of sulphate ions was analyzed by using barium chloride test.The resulting precipitate was dried by exposing it with UV light for 2 hrs. The dried powder was pre-sintered at 300oC for 2 hrin muffle furnace. The pre-sintered powder was milled in agate mortar with acetone base for 1 hr and sintered at 600oC for 4 hrs. Finally sintered powder was milled in agate mortar using acetone.

The structural properties of prepared ferrite were investigated by using X ray, FESEM, EDAX and FTIR tools. The crystalline phase of ferrite nanoparticles was determined by X-ray diffractometer (Philips EXPERT MPD), Cu-Kα (λ = 0.154 nm) source, in the range of 20–80o. The morphological study of the ferrite was done by using a FE-SEM (Zeiss Ultra 55 FE-SEM with Oxford EDX system). Perkin– Elmer Spectrophotometer (Model 783) was used to obtain FTIR spectrum of the ferrite.

The elemental analysis of ferrite material was carried out by using EDAX (Zeiss Ultra 55 FE-SEM with Oxford EDX system).

III. Result and discussion

3.1 XRD analysis

The X- ray diffraction pattern ofCu0.6Zn0.4Fe2O4 is shown in Fig. 1. The presence of (111), (220), (310), (311), (320), (400), (420), (421), (422), (500), (511), (440) and (442)peaks confirms the formation of single phase cubic spinel structure of desired ferrite. Lattice constant (a) of ferritewas calculated for most intense peak (311)using Bragg's formula and presented in the Table 1. It is observed that lattice constant (8.4471 Å) of the ferrite is slightly higher than reported for ceramic method [37]. This may be due to effect of synthesis technique of the ferrite under investigation.

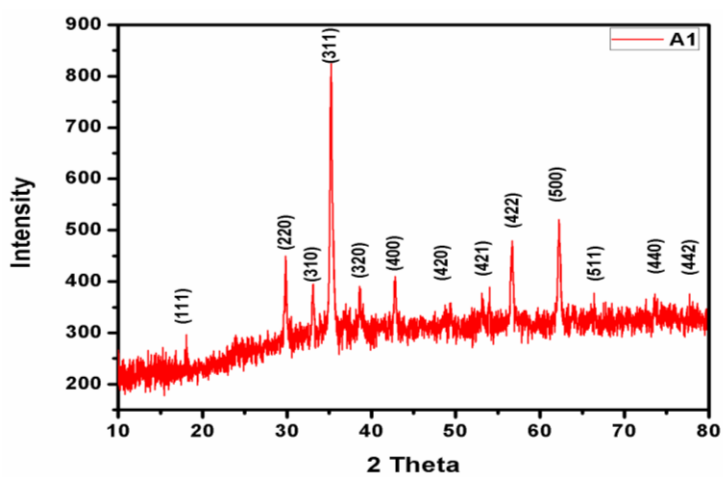

Fig. 1 – XRD pattern of Cu0.6Zn0.4Fe2O4 system

The X-ray density (ρx) of the ferrite was obtained by using the relation

$$\rho_x = \frac{8M}{Na^3} \qquad (1)$$

Where,

M is the molecular weight,

N is an Avogadro's constant

It observed that X- ray density (5.3022 gm/cm3) of the ferrite is 98 % higher than that of reported for bulk density[17, 18, 22, 38]. Li et al. [21] synthesized Cu-Zn ferrite by the sol-gel auto combustion method. They reported that the lattice constant and X-ray density of Cu0.6Zn0.4Fe2O4are 8.4273 Å and 5.3286 gm/cm3respectively. These values are closely agreed with our resulting values.

The crystallite size (D) of Cu0.6Zn0.4Fe2O4 was calculated for (311) plane using Debye Scherrer formula [40].

$$\mathbf{D = \frac{0.94\lambda}{\beta\cos\theta}} \qquad (2)$$

Where λ–Wavelength of X- ray,
θ- Braggdiffraction angle
β-full width at half maximum (FWHM).

The bond lengths (A-O, B-O), ionic radii (rA, rB) [41] and hopping lengths (LA, LB) [42] on tetrahedral (A) and an octahedral(B) sites of the ferrite were calculated by using the following relations

$$\mathbf{A - O = \left(u - \frac{1}{4}\right) a^{1/2}} \qquad (3)$$

$$\mathbf{B - O = \left(\frac{5}{8} - u\right) a} \qquad (4)$$

$$r_A = \left(u - \frac{1}{4}\right) a\sqrt{3} - r_0 \qquad (5)$$

$$r_B = \left(\frac{5}{8} - u\right) a - r_0 \qquad (6)$$

$$L_A = a\sqrt{\frac{3}{4}} \qquad (7)$$

$$L_B = a\sqrt{\frac{2}{4}} \qquad (8)$$

Crystallite size (D), bond lengths (A-O, B-O), ionic radii (rA, rB)and hopping lengths (LA, LB) of Cu0.6Zn0.4Fe2O4are presented in the Table 1. From table it is seen that the crystallite size of the ferrite lies in the nano-crystalline range (24.54 nm). It is revealed that the crystallite size of the ferrite is lower than that reported for ceramic [2, 37, 39] and sol-gel auto-combustion method[19, 21, 27, 40,43, 44,].This is attributed to effect of sintering temperature and duration followed during the preparation of ferrites.In ceramic method ferrites are obtained at higher sintering temperature (greater than 800 0C, 4 to 12 hr) [4, 34, 37, 38, 45, 46] whereas in sol-gel auto combustion method ferrites sintered near about 10000C for 4hr[44], 6500C for 4.5 hr [43], 14000 C for 2 hr [47] and 7500 C for 5hr [48]. In present work, it is clear that ferrite can be synthesized at lower sintering temperature as compared to othertechniques.

The bond length and the ionic radii on B site are greater than that of A site(table 1) and which is expectedsince octahedral site is larger than tetrahedral site. Also hopping length of A site is greater than that of B site (table 1).

Table 1: Structural parameters obtained from X- ray data of $Cu_{0.6}Zn_{0.4}Fe_2O_4$ system.

Lattice constant a (Å)	Crystallite size D (nm)	X-ray density ρ_x (gm/cm³)	Ionic radii (Å)		Bond length (Å)		Hopping length (Å)	
			r_A	r_B	A-O	B-O	L_A	L_B
8.4471	24.54	5.3022	0.598	0.740	1.918	2.060	7.315	5.973

3.2 FTIR analysis

Fig. 2 shows the FTIR spectrum of Cu0.6Zn0.4Fe2O4system. The presence of characteristic bands υ1 and υ2 near 600 cm–1 and 400 cm–1corresponding to tetrahedral and octahedral complexes confirms the formation of spinel ferrites[20].The band at 550.75 cm-1 is assigned to the vibrations between the tetrahedral metal ion and the oxygen ion (Mtetra-O) and the position at around 428.95 cm-1 is assigned to the vibrations of the band between octahedral metal ion and oxygen ion (Mocta-O). [16,23]. Similar results are reported by Guner et al. [19] for Cu-Zn ferrite prepared by citric acid assisted sol–gel auto combustion process. The appearance of band position at 379.455 cm-1may be due presence of copper ions at octahedral sites. Such types of band positions are also observed in the spectra of Cu-Zn ferrites prepared by standard double sintering ceramic method [49].

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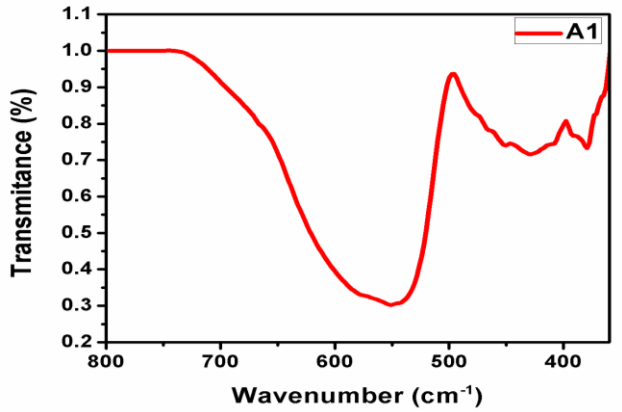

Fig. 2 –FTIR spectrum of Cu0.6Zn0.4Fe2O4 system

3.3 FESEM

The micro-photograph of Cu0.6Zn0.4Fe2O4system is presented in Fig. 3. Photograph clearly showsthe formation of spherical grains and confirms that the sintering temperatures as well as sintering period are sufficient to prepare ferrite.The grain size of the ferrite was calculated by using the linerintercept method and isabout 50.66nm. It is observed that crystallite size and grain size of the ferrite lies in nano-scale range. This indicates that the co-precipitation methodyields nano-size ferrite material at lower sintering temperature.It is reported that grain size of the ferrites prepared by ceramic method lies in the micrometer range [2, 37, 17,] whereas that prepared by chemical methods lies nearly above hundreds of nanometer range [43, 40, 44].

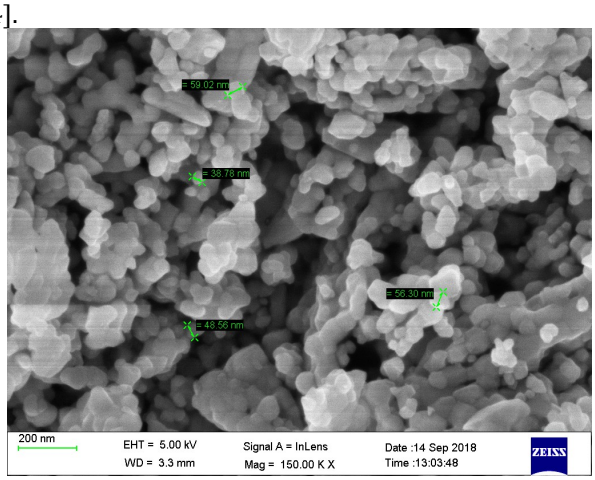

Fig. 3 – Field Emission Scanning Electron Microscopy of Cu0.6Zn0.4Fe2O4

3.4 EDAX analysis

The EDAX of Cu-Zn ferrite with composition Cu0.6Zn0.4Fe2O4ispresented in Fig. 3. From this spectrum, it is clear that there is a formation of desired ferrite in required stoichiometric ratio. It is also confirms that there is no any impurity phase observed in the spectrum. The elements present in the ferrite system according to weight % and atomic % are tabulated in table no. 2.

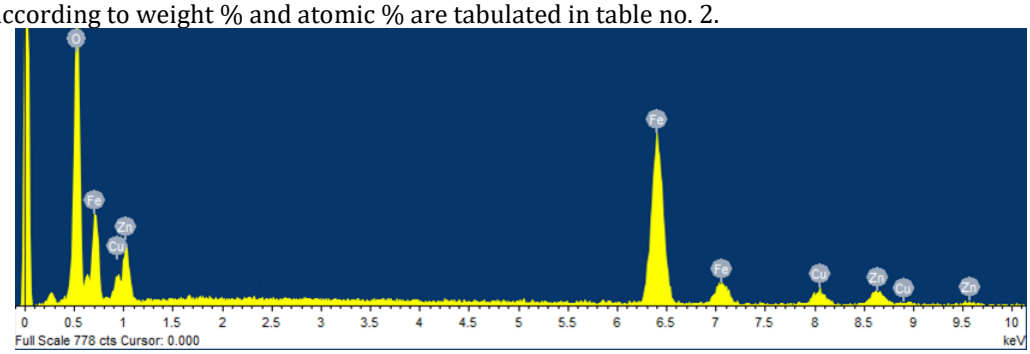

Fig. 4 – EDAX spectrum ofCu0.6Zn0.4Fe2O4

Table No. 2 – Weight % and atomic % of elements obtained by EDAX

Element	Weight %	Atomic %
O K	30.82	62.05
Fe K	45.45	26.23
Cu K	9.60	4.86
Zn K	13.94	6.86
Total	100	100

Conclusion

Nano-size copper-zinc ferrite material with composition $Cu_{0.6}Zn_{0.4}Fe_2O_4$ was successfully synthesized by oxalate co-precipitation technique at lower sintering temperature. X- ray diffraction analysis confirms the formation of single phase cubic spinal structure without any impurity phase.The presence of two absorption bands near about 400 $cm^{-1}$and 600 $^{cm-1}$ in FTIR spectrum confirms the well formation of spinel nano-ferrite. Microphotographs obtained from FESEM shows formation of nano-ferrite material. EDAX shows,resultant ferrite material obtained in required stoichiometric proportion.

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SYNTHESIS AND CHARACTERIZATION OF ZINC FERRITE BY MODIFIED CHEMICAL DEPOSITION METHOD

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ABSTRACT: *A modified low temperature chemical deposition strategy is employ for the preparation of nanocrystlline zinc ferrite thin film on pre-cleaned glass substrate. Such deposition method is relatively less pricey and convenient for large area deposition. The crystal structural and chemical structure analysis of zinc ferrite thin films is investigated by X-ray diffraction and FTIR spectroscopy techniques respectively. XRD study clearly shows the formation of cubic spinel structure.*

Keywords: *Deposition, Zinc ferrite, FTIR Spectroscopy, XRD, Cubic Spinel Structure*

I. Introduction

Materials science in a primitive form is one of the oldest forms of engineering and applied science. In other terms, materials science is defined as the one which is concerned with the relation of composition, structure, processing of materials, their properties and uses. Chemistry plays a fundamental role in interdisciplinary field i.e. material science. Study of this field helps to correlate the chemical and physical properties of thin films and thus in solving variety of problems of practical and technological importance, it also offers to synthesize material having improved properties of thin films provides base for developing new applications. This branch of science has been utilized in studying the various aspects of materials as synthesis, characterization and applications to fulfill the need of human being.

To face upcoming challenges in twenty first century, we require the miniaturization of device in to nanometer sizes when their ultimate performance gets dramatically enhanced. In 1959, Richard Feynman said that, " There is plenty of room at the bottom" and introduced for the first time concept of nanotechnology. He suggested that the manipulation of individual atom could be revolutionary to science. In recent years, nonmaterial's have attracted great attention from chemists, physicists and technologists due to their unique combinations of mechanical, thermal, electronic, optical, magnetic, and chemical properties. The prefix 'nano' in the world of nanotechnology means a billionth ($1x10^{-}9$) part. Nanotechnology deals with various structures of matter having dimensions of the order of a billionth size. The main emphasis on the synthesis, characterization and study of the variations in properties finds application in favor of human kind [1-4]. It is mentioned that, ' the small is beautiful' due to shape, color and properties of nanosized materials [5,6]. Fundamentally, the nanoscience and nanotechnology are the parts of the material science. The nanosized materials are distinguished from bulk polycrystalline materials by the size of crystallite that composes it. When dimension of the particles is smaller than 100 nanometers, it is termed as nanomaterial. These are not only interesting from the scientific point of view but also hold great potential for varied applications. A nanocrystalline material might be ceramic. Metallic oxide, semiconducting material or alloy

Ferrite may be defined as magnetic material composed of oxide containing ferric ions as the main constituents along with other divalent or trivalent metal ions. Ferrite materials have along history in their preparation and applications.

Types of ferrite

(i) Soft Ferrites
(ii) Hard Ferrites
(iii) Spinel Ferrites
(iv) Garnet ferrites
(v) Magnetoplumbite ferrites

Modified Chemical Deposition Method (M-CDM) :

In this modified chemical deposition method (M-CDM), thin films are obtained by immersing substrate into separately placed cationic and anionic precursors (rising between every immersion with distilled water is an option). The collection of (ions) substance on the surface of another substance is known as adsorption. [7]

II. Experimental setup

Fig.1.exhibits schematic experimental set-up used for the deposition of the NiFe2O4 thin films. M-CDM setup consists of two containers for both precursors, usually a glass beaker, fixed in a water bath. The water bath is kept on the magnetic stirrer equipped with temperature control set up in order to maintain the desired temperature using temperature controller Selec DC-204. The substrates were alternately immersed in beakers containing the precursor solutions.

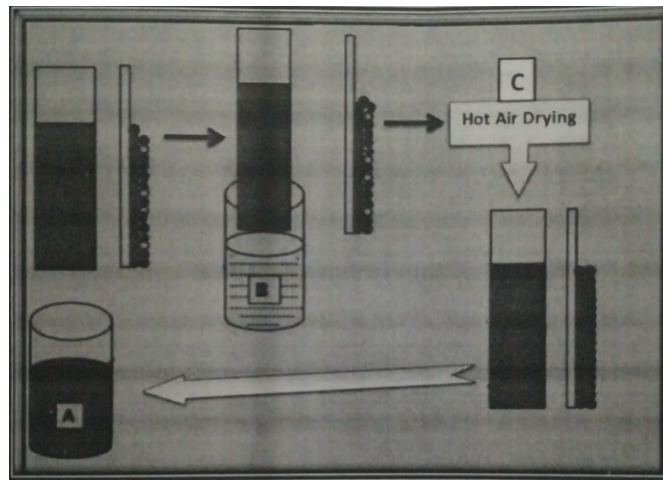

Fig.1. Schematic presentation of M-CDM

Define abbreviations and acronyms the first time they are used in the text, evenafter they have been defined in the abstract. Abbreviations such as IEEE and SI do not have to be defined. Do not use abbreviations in the title or heads unless they are unavoidable.

Nature and cleaning of substrate

For gas sensing the ZF thin films were deposited on the glass substrates. Substrate cleaning plays in important role in the deposition of thin films. Extreme cleanliness of the substrate was required for M-CDM because, contaminated surface provides nucleation sites facilitating growth resulting into non-uniform films with different orientation and impurities. The glass micro slides of dimensions 75 mm x 10 mm x 1.35 mm have been used as the substrates. The following procedure has been adopted for cleaning the substrates :

[1] The glass slides were washed with detergent and distilled water,
[2] Then dipped in concentrated chromic acid for 5 hours,
[3] The substrates were washed with double distilled water.
[4] Then substrates were ultrasonically cleaned for 15 min, and
[5] Finally, the substrates were dried, rinsed in AR grade acetone and were used for deposition.

2.1 Synthesis of zinc ferrite thin films :

The deposition of ZF thin films was done on micro glass slides. Two-beaker system was used for the deposition onto the glass substrate by alternate immersion of substrate in cationic precursor and anionic precursor. The cationic precursor contains Ni^{2+} and Fe^{2+} in 1:2 proportions. The aqueous ammonia solution (29%) was used to adjust the alkaline pH of cationic precursor. It acts as complexing agent. First, the ultrasonically cleaned glass substrate was immersed in cationic precursor for 30 seconds termed as Adsorption period, so as to get zinc and iron hydroxides adsorbed onto the substrate. Double distilled water was used as anionic precursor maintained at 27° C Afterwards the substrate was rinsed with anionic precursor for 30 seconds termed as Reaction period. During reaction period the oxygen ions reacted with pre-adsorbed hydroxides species. These thin films were dried by hot air after each cycle, which results into oxidation of some of the Fe^{+2} To Fe^{+3} along with that iron ox hydroxide (goethite) also formed as required to form ZF [8] This completes one deposition cycle; such types of numbers of depositon cycle were required for the ZF thin films. These thin films were air annealed at 450 ° C for 5 hours to form ZF thin film with cubic spinel phase by removing any hydroxide content. Fig.2. shows (A) as prepared and (B) annealed ZF thin film. The Ni Fe2O4 thin film prepared.

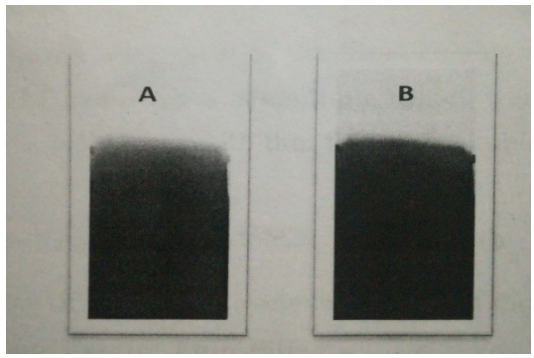

Fig.2. ZF thin film (A) as prepared and (B) annealed

All the preparative parameters for the ZF thin films of various compositions are listed in following table.1.

Table .1.Preparative parameters of ZF thin films

Compositions of thin films	pH	Bath temperature (°C)	Deposition Cycles	Adsorption and reaction period (Sec)
ZnFe2o4	9.50	55	42	30

Mechanism of film formation

The reaction of ZF thin films formation by M-CDM is represented by the following reaction First, the ultrasonically cleaned glass substrate was immersed in cationic precursor for 30 sec, so as to get zinc an iron hydroxides adsorbed onto the substrate. The reactions takes place same as,

$$ZnCl_2 + 2NH_4\,OH \rightarrow Zn(OH)_2 + 2NH_4Cl$$
$$FeCl_2 + 2NH_4OH \rightarrow Fe(OH)_2 + 2NH_4Cl$$

Afterwards the substrate was rinsed with anionic precursor (double distilled water kept at 27 °C, for 30 sec, during reaction period the oxygen ions reacted with pre-adsorbed hydroxides of zinc and iron on the glass substrate and also to remove loosely binded hydroxides species. These thin films were dried by hot air after each cycle which results into oxidation of some of the Fe^{+2} to Fe^{+3} along with that, iron ox hydroxide (geothite) also formed as required to formed as required to form ZF[8,9].

$$Fe(OH)_2(OH)\_2 \xrightarrow{O2(Hol.Air)} FeOOH + H\_2\,O\uparrow$$

Hot air drying improved adherence of the film. This completes one deposition cycle of ZF thin films by M-CDM. Such types of multiple cycles were carried out. Finally, films were air annealed at 450 °C, to form pure ZF thin films with cubic spinel phase by converting hydroxide content.

$$Zn(OH)_2 + 2FeOOH \xrightarrow{450°c} ZnFe_2O_4 + 2H_2O\uparrow$$

Changing the number of deposition cycles thickness of the film was controlled

3. Results and Discussions

3.1 X-ray diffraction study :

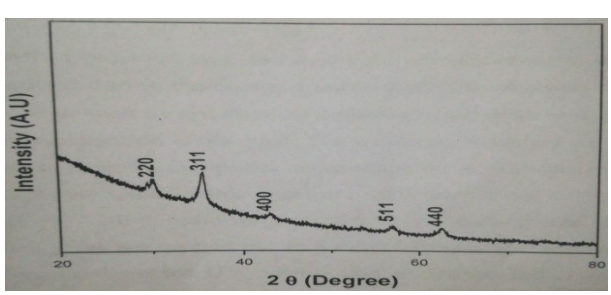

Fig.3.X-ray diffraction pattern of ZF thin films

X-ray diffraction technique was used to investigate the structural identification, lattice parameters determination, phase and crystallite orientation of the material. Fig.3.shows the X-ray diffraction pattern of ZF thin films annealed at 450 °C for 5 hours. The sharp peaks indicate that, the well crystallized nature of ferrite. The XRD pattern oriented with high intense (311) plane which is the characteristic peak of the spinel

phase of ZF thin films. Other orientations corresponding to (220), (400), (511) and (440) planes are also present with relatively lower intensities compared to that of (311) plane are in best agreement with the cubic spinel (JCPDS card no:74-2397). This confirms that zinc ferrite phase has been formed.

3.2 FT-IR Spectroscopy analysis

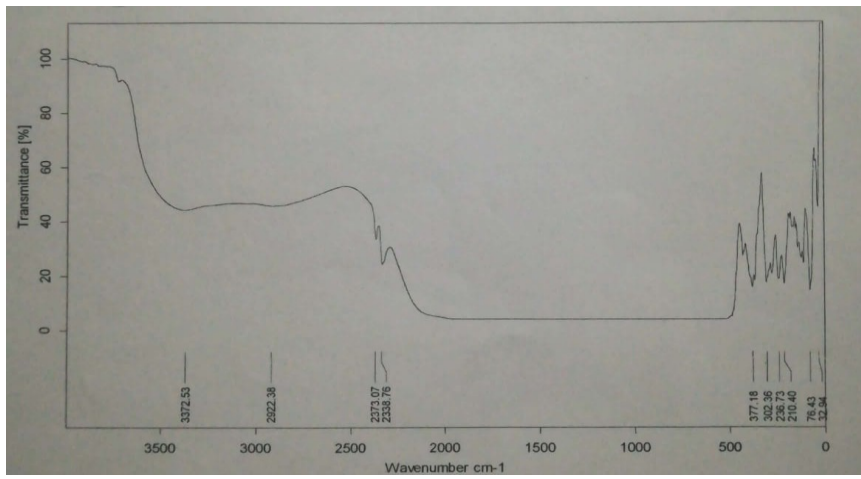

Fig.4.FTIR spectroscopy is used to detect the composition of the solid state reaction and study metal ion distribution in the material. Fig.4.shows the FT-IR spectrum of annealed ZF thin film. These spectra are taken in KBr, with sample to KBr ratio 1:300. In case of ferrite, two bands are observed around 400 cm^{-1} and 600 cm^{-1}. There are observed values of octahedral band at lower frequencies 377 cm^{-1} due to vibration of M-O group and tetrahedral frequencies at around 577 cm^{-1} (hide due to peak broadening & can be separated by curve fitting). Thin film has tetrahedral peak and weak octahedral peak. This confirms stable oxide of Zinc ferrite formed.

4. Conclusion:

A modified chemical deposition method is utilized for the synthesis of nanocrystalline zinc ferrite thin films onto glass substrates at low temperature i.e 55°C temperatures. This method is relatively less expensive and convenient for large area deposition of metal oxide films. Nanocrystalline Zine ferrite films have been deposited onto the glass substrates using zinc (II) Chloride and iron (II) chloride as cationic precursors. Double distilled water was used as an oxidizing agent. The structural and infrared characterizations of the films were studied using X-ray diffraction (XRD) and FT-IR spectroscopy. The results show that modified chemical deposition method (M-CDM) is successfully employed for the preparation nanocrystalline $ZnFe_2O_4$ thin films with spinel phase.

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X-RAY DIFFRACTION ANALYSIS OF Ni-Cu-Zn NANO-FERRITE SYNTHESIZED BY WET CHEMICAL ROUTE

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ABSTRACT: *Ni-Cu-Zn nano-ferrite with composition $Ni_{0.7}Cu_{0.1}Zn_{0.2}Fe_2O_4$ was synthesized by wet chemical rout. The structural parameters such as lattice constant (a), crystallite size (D), bond lengths (A-O, B-O), ionic radii (r_A, r_B), X- ray density (ρ_x), hopping lengths (L_A, L_B) were obtained from X- ray diffraction analysis. The presence of allowed planes in the X- ray diffraction pattern confirms the formation of single phase cubic spinel structure. It was found that the values of lattice constant and X-ray density of the ferrite are similar than that reported for ferrite prepared by citrate precursor method followed by microwave sintered technique. Crystallite size of the ferrite lies in nano-size range and which is much lower than that reported for ferrites prepared by ceramic as well as citrate precursor methods. Bond length (B-O) and ionic radii (r_B) on octahedral site are higher than that of observed for tetrahedral site. Hopping length of ferrite on tetrahedral (A) site is higher than that of octahedral (B) site.*

Keywords: *nano-ferrite; wet chemical route; Ni-Cu-Zn ferrite; X-ray diffraction*

1. Introduction

Recently researchers in different fields are engaged in the development of nano-materials in the form of nano-ferrites. A nano-ferrite material has excellent and improved properties as compared to that of reported for bulk materials. These materials are technologically important and used in many applications such as including magnetic recording media and magnetic fluids for the storage and or retrieval of information, magnetic resonance imaging (MRI) enhancement, magnetically guided drug delivery, catalysis, sensors and pigments [1-3]. Recently instead of Ni-Zn and Mg-Zn nano-ferrites, there is a growing interest on the synthesis of copper substituted nano-ferrites because of its growing applications.

Various chemical methods such as reverse micelle method, auto-combustion method, oxalate based precursor method, microwave sintering method, sol-gel method etc were used to prepare Ni-Cu-Zn nano ferrites. Ghasemi et al. [4] prepared copper substituted Ni-Zn nano-crystalline ferrites by reverse micelle process. They reported that the saturation magnetization of Ni-Zn ferrites decreases with increasing copper content. Ni-Cu-Zn nano-ferrites prepared by auto-combustion method utilized for the fabrication of multilayer chip inductor [5]. Raghavender et al. [6] studied structural and dielectric properties of Ni-Cu-Zn ferrites synthesized by oxalate precursor method. They reported that the dielectric constant and loss of these ferrites are lower than that of reported by other synthesis methods. The structural, magnetic and electrical properties of Ni-Cu-Zn ferrites followed by microwave sintering technique have been reported by Reddy et al. [7]. They revealed that ferrite material obtained by microwave technique has improved electro-magnetic properties. They also suggested that these ferrite materials are suitable for the fabrication of multilayer chip inductors used in the electronic devices.

In present communication, we discuss structural parameters of Ni–Cu-Zn ferrite prepared by wet chemical method.

2. Experimental

2.1 Synthesis of Ni-Cu-Zn ferrite.

Ni-Cu-Zn nano-ferrite with composition $Ni_{0.7}Cu_{0.1}Zn_{0.2}Fe_2O_4$ was prepared by wet chemical method using sulphates as the starting materials. AR grade ammonium oxalate was used as a precipitating reagent. The required sulphates were weighed in desired proportion with the help of higher accuracy digital micro-balance and poured in the double distilled water. The dropwise conc. sulphuric acid was added in the solution of mixture with continuous stirring. The magnetic stirrer was used for stirring. Ammonium oxalate solution was added in the solution until precipitation process was completed. The precipitated solution was filtered and washed several times. The precipitate was dried and pre-sintered at 400^0C for 2 hours. The pre-

sintered powder was milled and finally sintered at 600 ^{0}C for 4 hours. Pre-sintering and sintering process of the material was carried out in auto controlled muffle furnace.
The resulting ferrite powder was characterized by X-ray powder diffractometer of Phillips makes Model 3710 with Cu-Kα radiation.

3. Result and discussion

3.1 XRD Analysis

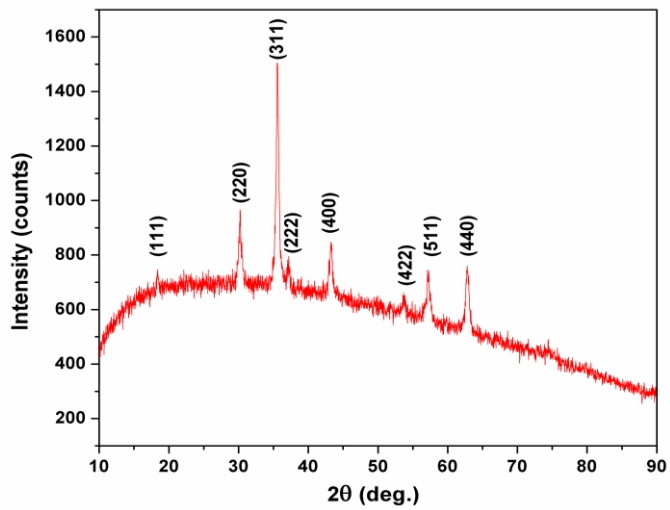

Fig. 1. X- ray diffraction pattern of $Ni_{0.7}Cu_{0.1}Zn_{0.2}Fe_2O_4$ system

Fig. 1 shows XRD pattern of $Ni_{0.7}Cu_{0.1}Zn_{0.2}Fe_2O_4$ system. The presence of (111), (220), (311), (222), (400), (422), (511) and (440) planes in the pattern confirmed the formation of single phase cubic spinel structure. The positions of allowed planes are well match with those reported for Ni-Cu-Zn ferrite prepared by soft chemical method [8].
The lattice parameter 'a' for most intense (311) peak was calculated using Bragg's formula and it is found to be about 8.362 Å. It is seen that the lattice constant of the ferrite prepared under investigation is nearly same as that of synthesized by citrate precursor method carried out under microwave sintering technique [9] and is also slightly lower as compared to that reported by Ramkrishna et al. [10] for ferrite prepared by co-precipitation method.
The calculated and observed interpleader distances (d_{cal} and d_{obs}) of $Ni_{0.7}Cu_{0.1}Zn_{0.2}Fe_2O_4$ with diffraction angles and planes are presented in Table 1. From this table, it is seen that d_{cal} and d_{obs} values of each plane are approximately matched with each other and support for the confirmation of single phase cubic structure. Crystallite size of the ferrite was calculated for most intense (311) plane by using Debye Scherer formula (Table 2). It is observed that crystallite size of the ferrite found to be lower as compared to that reported for citrate precursor method [11], sol-gel auto combustion method [12] and Co-precipitation method [13]. It is clear that with wet-chemical method well nano-size ferrite can be prepared.

Table 1 Inter-planer spacing's of $Ni_{0.7}Cu_{0.1}Zn_{0.2}Fe_2O_4$ system

Angle 2θ (deg.)	**Angle Θ (deg.)**	**hkl planes**	**Inter-planer spacing (Å)**	
			d_{obs}	**d_{cal}**
18.355	9.177	111	4.8391	4.8335
30.214	15.107	220	2.9633	2.9580
35.523	17.761	311	2.5271	2.5272
37.158	18.579	222	2.4195	2.4196
43.305	21.652	400	2.0954	2.0894
53.739	26.869	422	1.7108	1.7058
57.188	28.594	511	1.6130	1.6108
62.795	31.397	444	1.4816	1.4786

The X-ray density (ρ_x) of the ferrite was obtained by the following relation [14]

$$\boldsymbol{\rho_x = \frac{8M}{Na^3}} \quad \text{---------- (1)}$$

Where,

M is the molecular weight,
N is an Avogadro's no.
a is lattice parameter

X-ray density of the ferrite is found to be about 5.3656 gm/cm^3 and is nearly same as that of reported by Thangjam and soibam [9] for Ni-Cu-Zn nano-particles prepared by citrate precursor method.

Bond lengths (A-O, B-O) and ionic radii (r_A, r_B) on A-site B- site are calculated by using Standley's relations [14]. The hopping lengths on tetrahedral (A) and an octahedral (B) sites (L_A & L_B) are calculated by using the relations suggested by Chaudhari, et al. [15].

The tetrahedral (dAe), octahedral (dBe) and unshared octahedral (dBeu) edges are calculated by using the relations [16].

$$\text{dAe} = \boldsymbol{a\sqrt{2}\,(2u - 0.5)} \quad \textbf{(2)}$$

$$\text{dBe} = \boldsymbol{a\sqrt{2}\,(1 - 2u)} \quad \textbf{(3)}$$

$$\text{dBeu} = \boldsymbol{a(4u^2 - 3u + 0.73)^{1/2}} \quad \textbf{(4)}$$

Structural parameters such as, crystallite size (D), X-ray density, bond lengths (A-O, B-O), ionic radii (r_A, r_B), hopping lengths (L_A and L_B), tetrahedral edge (dAe), octahedral edge (dBe) and octahedral unshared edge (dBeu) are presented in Table 2

Table 2 XRD structural parameters of $Ni_{0.7}Cu_{0.1}Zn_{0.2}Fe_2O_4$ system

Crystallite size D (nm)	X-ray density ρ_X (gm/Cm^3)	Ionic radii (A^0)		Bond length (A^0)		Hopping length (A^0)		Tetrahedral, octahedral and unshared edge (Å)		
		r_A	r_B	A-O	B-O	L_A	L_B	dAe	d_{Be}	d_{Beu}
21.2	5.3656	0.583	0.724	1.903	2.044	7.258	5.926	3.101	2.812	3.424

From table 2. it is observed that bond length (A-O) and ionic radii (r_A) on A- site is less than that of B-sites. Similar type of result was reported by Shinde et al. [14] for Ni-Zn ferrites synthesized by co-precipitation method. Hopping length on A- site is greater than that of B- site. It may be due to the presence of more divalent metal ions on A- site than that of B- site.

4. Conclusion

By wet-chemical method $Ni_{0.7}Cu_{0.1}Zn_{0.2}Fe_2O_4$ nano-material was easily synthesized at lower sintering temperature and smaller duration. The formation of single cubic spinel structure was confirmed by X-Ray diffraction analysis. The crystallite size of the ferrite is about 21.2 nm and is much smaller than that reported for other methods. It is seen that lattice constant of the ferrite is nearly same as that of reported for citrate precursor method followed by microwave sintering technique. Bond length and ionic radii on A-site are smaller than that of B-site

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SYNTHESIS, CHARACTERIZATION AND ELECTROCHEMICAL PERFORMANCE OF NANOSTRUCTURED V_2O_5 THIN FILM DEPOSITED BY HYDROTHERMAL METHOD

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ABSTRACT: *In the present study, vanadium pentoxide (V_2O_5) thin films have been successfully deposited by a simple hydrothermal method at 180°C. These thin films were characterized for thermal, structural, morphological and surface wettability by using TGA-DTA, X-ray diffraction (XRD), Fourier transformation Raman (FT-Raman) spectroscopy, scanning electron microscopy (SEM) and contact angle measurement techniques. The electrochemical capacitor performance was examined by using cyclic voltammetric and galvanostatic charge-discharge method. The specific capacitance 286 F/g was obtained in 1 M Na_2SO_4 solution within the potential range of 0.2 V to 0.6 V versus SCE. The supercapacitor exhibited a good cycling performance and can be used to fabricate supercapacitor device.*

Keywords: *V_2O_5; hydrothermal method; thin film; supercapacitor.*

1. Introduction

Supercapacitors, are known as a new generation of electronic devices and have attracted much attention because of their simple principle, long cycle life, pulse power supply and high dynamic of charge propagation [1-2]. Supercapacitors are classified mainly into two types; first one electric double layer capacitor (EDLC) which stores charge electrostatically or non-faradaic and there is no transfer of charge between electrode and electrolyte. Second type is pseudo capacitor in which charge storage is faradaic through the transfer of charge between electrode and electrolyte [3]. Hybrid capacitor is the combination of EDLC and pseudocapacitor in which charge is stored using both faradaic and non-faradaic processes.

Vanadium oxides (V_2O_5) have been widely examined as an electrode material for electrochemical capacitors that use organic electrolytes. Vanadium also exhibits numerous oxidation states (II–V), but its poor electronic conductivity ($\sigma_{bulk} \approx 1x10^{-4}\Omega^{-1}m^{-1}$) renders the oxide unsuitable for use in high-rate electrochemical devices. V_2O_5 is the most attractive and promising material because of its layered structure [4-5] and received a research interest due to its potential applications in lithium ion batteries [6-8], catalysts [9-11], electrochromic devices (ECDs) [12-14] and supercapacitor [15-17]. V_2O_5 thin film is a promising material because of its high specific capacitance. Up to now V_2O_5 has been synthesized by different synthesis methods such as chemical vapor deposition [18-21], sol gel method [22], electrochemical deposition [23] and hydrothermal [24-25] etc. In present study, the hydrothermal method is used because hydrothermal is simple, inexpensive method is a low temperature (180°C) and a short reaction time (3 h) as compared to the conventional hydrothermal method. This technique to prepare V_2O_5 thin film using NH_4VO_3 as an initial material and HNO_3 pH control and this process needs no stirrer and surfactance. Neutral Aqueous electrolytes rechargeable supercapacitors have attracted much more attention because of their advantages like high ionic conductivity, low-cost, non flammability, no specific requirements for battery assembly and good safety [26-27]. V_2O_5 material has large theoretical capacity of 294 fg^{-1}, [28] which is rather higher than that of commonly used cathode materials, like $LiCoO_2$ (140 fg^{-1}), $LiMn_2O_4$ (148 fg^{-1}) and $LiFePO_4$ (170 fg^{-1}).

In the present work, V_2O_5 thin films are successfully deposited by a simple, inexpensive and novel hydrothermal method. The thin films are characterized for thermal, structural, surface morphological and wettability studies. The electrochemical properties of V_2O_5 thin film are examined using cyclic voltammetry (CV). Galvanstatic charge-discharge. The electrochemical reaction of V_2O_5 in neutral aqueous electrolyte is elucidated by analyzing the electrochemical behavior of V_2O_5 in 1 M Na_2SO_4 and their corresponding structure & composition changes during charge-discharge. Also V_2O_5 thin films are successfully deposited by hydrothermal reduction of NH_4VO_3 as an initial material. The thin films formation mechanism of orthorhombic V_2O_5 nanostructure is briefly discussed.

2. Experimental

The commercially available AR grade ammonium metavanadate (NH_4VO_3) (98.0 % Loba Chemie Pvt. Ltd.), nitric acid (HNO_3) (69-72 % Loba Chemie Pvt. Ltd.) and sodium sulfate (Na_2SO_4) (99.0 % Loba Chemie Pvt. Ltd.) are used as received without further purification. All the chemicals are prepared in double distilled water. V_2O_5 thin films are deposited on stainless steel substrate surface from a bath of NH_4VO_3 solution. For deposition of good quality thin films the experimental conditions, such as pH of the reaction bath, reaction temperature and duration of the hydrothermal treatment are examined. In a typical synthesis, an appropriate amount of 0.5 M HNO_3 solution is added to 25 ml of 0.05 M NH_4VO_3 solution, to adjust pH in the range of 2–3. The solution is placed into a 50 ml stainless steel autoclave with a teflon liner and maintained at temperature 180 °C for 3 h and then cooled to room temperature naturally. The as obtained thin film washed three times in deionized water and finally annealed at 450 ^{0}C in air for 3 h to get pure V_2O_5 thin films.

The thermal analysis tests (TGA & DTA) conducted in nitrogen atmosphere at a heating rate of 10^o C/min, from room temperature to 1000^o C using an SDT Q600 Build 20 instruments. X-ray diffraction (XRD) pattern of final product recorded in the 2θ rang of 10° - 80° by using Cu Kα radiation (λ= 1.5418A^o) (Bruker D-8 diffractometer). Raman study carried out in the spectral rang of 100-1200 cm^{-1} using Bruker RAM. The surface morphological study of thin film carried out by using scanning electron microscope (SEM) (JEOL model 6360). The water contact angle of V_2O_5 film surface was measured using digital Goniometer (Rame-Hart NRL CA). The cyclic voltammetry (CV) & galvanostatic charge discharge measurement carried out using automatic battery cycle (WBCS 3000). The electrochemical characterization carried out in three electrode cell configuration by using graphite as a counter electrode, saturated calomel electrode (SCE) as a reference electrode and & V_2O_5 thin film as a working electrode in 1 M Na_2SO_4 aqueous electrolyte.

3. Results and Discussions

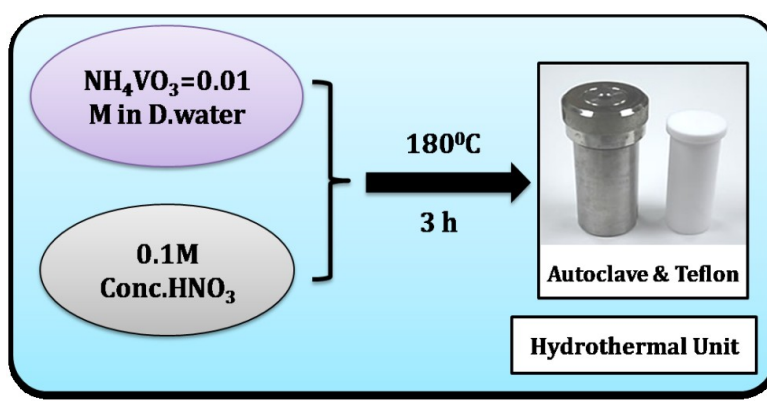

Fig. 1. Schematics of synthesis of V_2O_5 thin film by hydrothermal method

Fig.1. shows schematic of hydrothermal deposition of V_2O_5 thin film. The deposition is done by using aqueous solution of NH_4VO_3 in little amount of HNO_3 and pH is adjusted to ~2. The solution was put into a teflon liner with a stainless steel autoclave and cleaned substrate was immersed in to solution. The autoclave is put into a furnace and maintained temperature and after 3 h cooled down naturally. The process may involve chemical reaction (1).

$$2NH_4VO_3 + H_2O + HNO_3 \rightarrow V_2O_5 + 2NH_4NO_3 \text{-------(1)}$$

The thickness of the V_2O_5 thin film is calculated by a gravimetric method. The typical working electrode is 0.120 mg in weight with a surface area of 1 cm^2 and its thickness was 357 nm, which was calculated by using the formula (2)

$$t = \frac{m_2 - m_1}{\rho \times A} \text{---------------(2)}$$

Where, 'm_1' is mass of electrode material before the deposition and 'm_2' is mass of electrode material after deposition, 'ρ' is the density of electrode material (3.36 gcm^3), 'A' is active area of the electrode material deposited on the steel substrate (cm^2) and't' is thickness of thin film electrode (nm).

Fig.2. shows TGA-DTA results performed in nitrogen atmosphere and were also carried out to determine

potential reactions during heat treatment at a heating rate of 10° C/min. The loss of water from the precursor is attributed to the observed endothermic peaks. The V_2O_5 exhibits two weight loss slopes, first weight loss is up to 110°C due to the loss of weakly bound water and retained weight 10.85 %. The second wave of weight loss extends up to 15.62 % around 450°C, which is an indication of the thermal decomposition of the precursor.

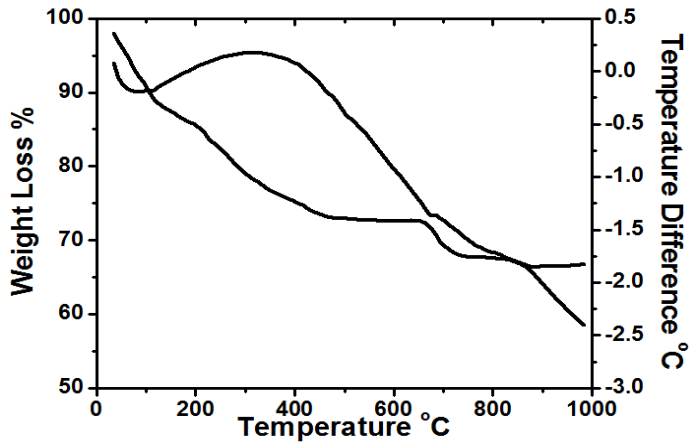

Fig. 2. TGA and DTA of (V_2O_5) thin film deposited by hydrothermal method

The formation of V_2O_5 phase is confirmed by an exothermic peak around 315° C in DTA spectrum. There is an endothermic peak around 675°C which may be due to the melting of sample and close to that of the reported melting point of V_2O_5.

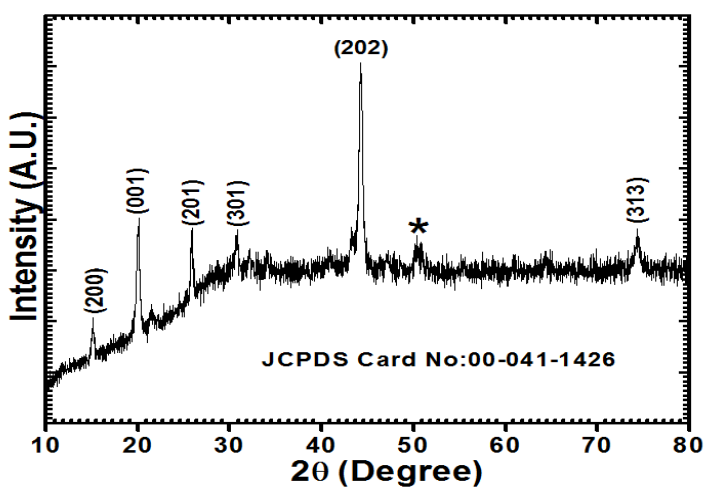

Fig.3. shows XRD pattern of V_2O_5 thin film on a stainless steel substrate.

It exhibit diffraction peaks along 2θ = 14.94, 19.77, 25.61, 30.93, 44.13, 74.59 degree and correspond to the lattice planes (200), (001), (201), (301), (202) and (313) respectively. The main diffraction peak indexed at 44.13° can be assigned to the (202) reflection. The diffraction peaks are in good agreement with standard JCPDS Card (no. 41-1426) of the V_2O_5 having orthorhombic crystal structure [29-38]. The * peak is due to the stainless steel substrate surface. The crystallite size is determining by using Scherer's formula (3)

$$D = K\lambda / \beta \cos\theta \text{ ---------(3)}$$

Where 'D' is crystalline size, 'K' is constant 0.9, 'λ' is wavelength of monochromatic X-ray (1.5418 Å) 'β' is full width at half maxima of the peak and 'θ' is diffraction angle. The crystallite size for (202) plane is found to be 24.21 nm.

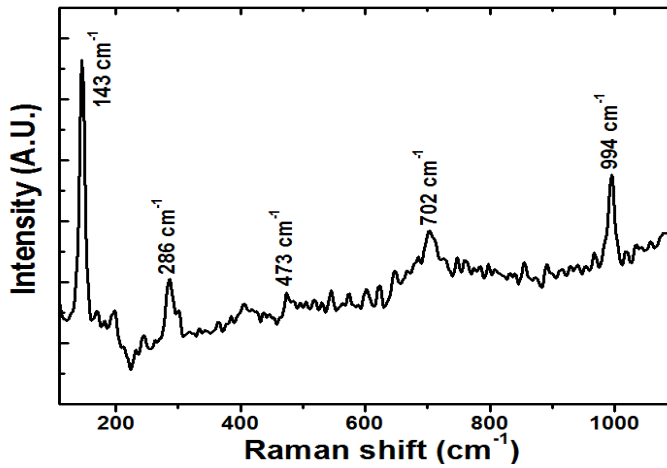

Fig..4. FT-Raman spectrum of V_2O_5 thin film deposited by hydrothermal method

The FT-Raman spectrum of V_2O_5 thin film is recorded over 110-1100 cm^{-1} and is shown in fig. 4. The Raman peaks observed in present spectrum at low and high frequency region. The spectrum shows well defined peaks at 143, 286, 473, 702 and 994 cm^{-1}. The most intense peak observed at 143 cm^{-1} is attributed to the skeleton bend vibration [39], while the narrow peak at 994 cm^{-1} corresponds to the terminal oxygen (V ≡ O) stretching mode [40]. Peak at 702 cm^{-1} is due to coordination of vanadium atom with the three oxygen atoms. The V-O-V stretching and bending modes are assigned to the frequency 286 cm^{-1} [41-42].

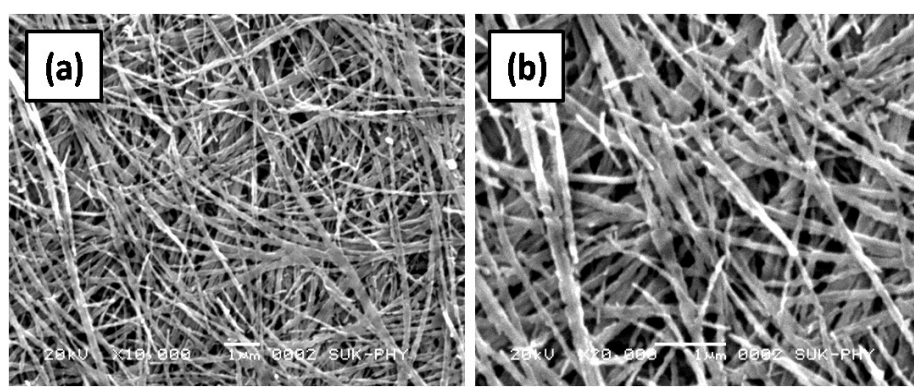

Fig.5. SEM images of V_2O_5 thin film with different magnifications

The surface morphological architecture of thin film is studied by using SEM. Figure 5 show high and low magnification images of V_2O_5 thin film. The SEM images show total coverage of substrate surface with V_2O_5 nanofibers. The fibrous networks have an average diameter of about 40 to 100 nm and length up to 6 - 7 µm. Such surface structure offer better performance in supercapacitor devices, because this surface provides efficiently minimum distance and time to travel charge carriers.

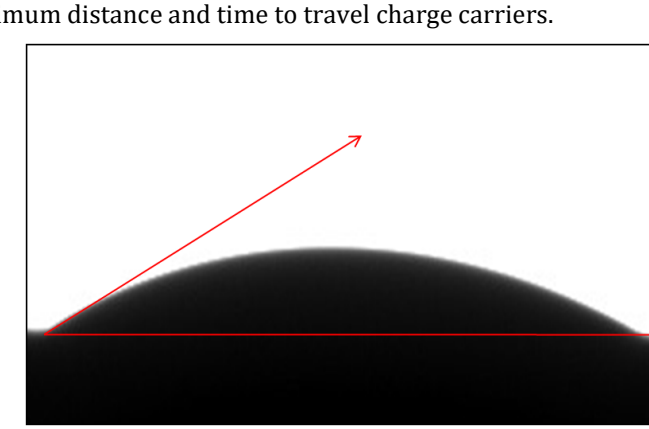

Fig.6. Contact angle image of V_2O_5 thin film surface (water droplet used as solvent)

Fig. 6. shows contact angle image of V_2O_5 thin film surface. Surface wettability test is investigated for the determination of hydrophilic ($\theta < 90^o$) or hydrophobic ($\theta > 90^o$) nature of thin film surface. If the wettability is high or low then strong or weak interaction between electrode and electrolyte solution refers to hydrophilic or hydrophobic nature of a V_2O_5 film surface. The V_2O_5 thin film exhibits contact angle of 20^o, which confirms super hydrophilic ($20^o > \theta$) nature. The super hydrophilic nature of film surface can give enhancement the electrochemical performance and good interfacial interaction between electrode electrolyte interfaces.

4. Supercapacitive performance

4.1. Cyclic voltammetry of V_2O_5 Thin Films

The hydrothermal deposited V_2O_5 thin film electrode is used in electrochemical capacitors and their performance is tested using cyclic voltammetry (CV). Fig.7. show cyclic voltammetry (CV) curve of V_2O_5 electrode at a scan rate of 10 mV/s within potential from -0.2 to +0.6 V vs. SCE in 1 M Na_2SO_4 electrolyte.

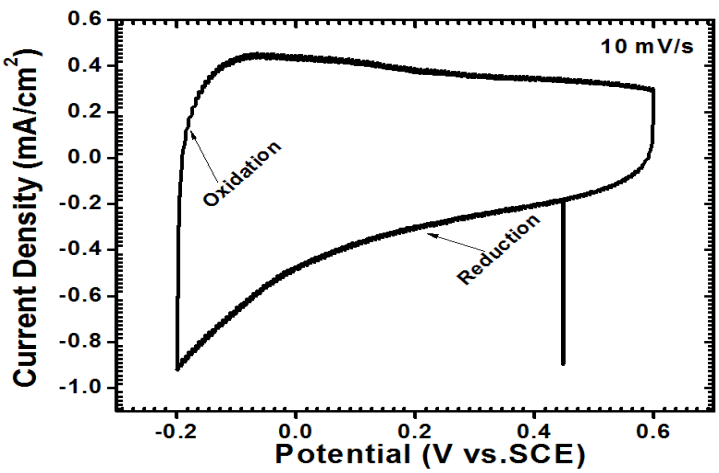

Fig.7. CV curves of V_2O_5 electrode in 1 M of Na_2SO_4 electrolyte

From shape of CV it is confirmed that capacitance arises from redox transition and EDLC within the electrode/electrolyte and electrolyte/electrode interface, respectively. The capacitance of film is calculated by using equation (4)

$$C = \frac{I}{dV/dt} \qquad (4)$$

Where 'I' is average current, 'dV/dt' is scanning rate of the electrode. The specific capacitance of the electrode is obtained by dividing the capacitance to weight (0.0001 g) dipped in the electrolyte. The specific capacitance of the electrode is found to be 286 F/g in 1 M Na_2SO_4 electrolyte.

4.2. Galvanometric Charge-Discharge Cycling Measurements

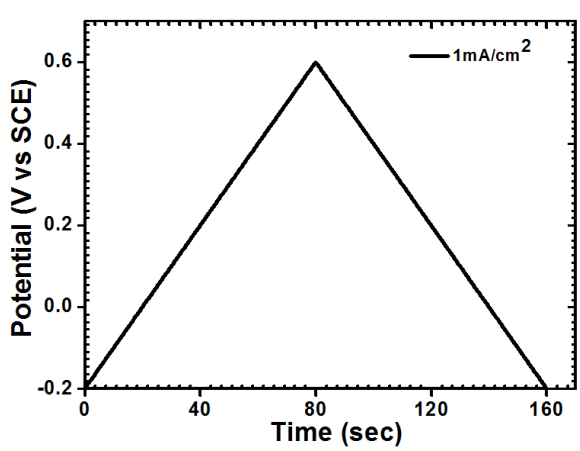

Fig.8. Charge discharge curve of V_2O_5 electrode in 1 M Na_2SO_4 electrolyte at current density of 1 mA/cm^2

The charge-discharge behavior of V_2O_5 electrode is studied by galvanometric charge-discharge method. Fig.8. represents charge-discharge behavior of electrode at current density of 1 mA/cm^2. The shape of the discharge curve does not show the capacitance characteristics of pure double layer capacitor. During discharging curves, linear variations of the time dependence of the potential (-0.2 to 0.6 V) indicate electric double-layer capacitance, which is the charge separation across the interface between the electrode and the electrolyte.

5. Conclusions

In summary, V_2O_5 thin films were successfully deposited onto stainless steel substrate by hydrothermal method. The XRD measurements confirmed that deposited V_2O_5 having orthorhombic crystal structure. The SEM analysis showed that the substrates were well covered with nanofibers. The electrochemical study revealed that the hydrothermal deposited V_2O_5 electrode had a specific capacitance of 286 F/g. Charge-discharge curves confirmed that capacitance was consisted from EDLC and pseudocapacitance. The hydrothermal method is a low cost and promisingly used for deposition of nanostructured thin film material for electrochemical supercapacitor.

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H_2S GAS SENSING PERFORMANCE OF UNDOPED CADMIUM OXIDE AND MIXED CADMIUM ZINC OXIDE ADVANCED SPRAY DEPOSITED THIN FILMS: A COMPARATIVE STUDY

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ABSTRACT: *Polycrystalline cadmium oxide (CdO) and cadmium zinc mixed oxide ($Cd_xZn_{1-x}O$) thin films were deposited onto glass at low substrate temperature using advanced spray pyrolysis (ASP) technique. A 0.02 M aqueous solutions of high purity cadmium acetate [Cd $(CH_3COO)_2$. $2H_2O$] (Thomas Baker, India) and an equimolar solution of zinc acetate [Zn $(CH_3COO)_2$. $2H_2O$] (Thomas Baker, India) were used as initial ingredients. The films were grown at optimized preparative parameters and the physical, morphological, optical & gas sensing properties of the films were investigated. X-ray diffraction (XRD) studies show that films thus prepared were polycrystalline in nature. Undoped CdO films have preferential orientation along (111) plane and mixed oxide films show shift in crystal structure with increase in zinc content. Scanning electron microscopy (SEM) micrographs revealed that the films have granular surface morphology. Optical properties were investigated using UV-VIS spectroscopy in the range 300 to 900 nm and was observed that films were transparent in nature. Typical samples of undoped and mixed oxide films were subjected to different concentrations of H_2S gas at different operating temperatures. The undoped CdO film showed maximum sensitivity ~ 4.8 % at while mixed oxide film showed maximum sensitivity around 34.2% when subjected to 200 ppm of H_2S gas.*

Keywords: *Undoped Cadmium Oxide; Mixed oxide: Advanced spray pyrolysis technique; surface morphology, Gas sensing.*

1.0 Introduction

Industrial revolution since nineteenth century has brought about drastic changes in the living standards of human population around the globe and has made it more comfortable. This has lead to extensive use of combustible gases like LPG, petrol and petroleum products over the years resulting into environmental hazards such as air pollution and global warming. Additionally, release of toxic gases like H_2S emerging from industrial waste is causing serious health problems to mankind. It is therefore inevitable to design and fabricate the technology in the form of devices comprising semiconductors, metal oxides etc. which can detect and display their concentrations in the environment during the early stages of release. Recently, thin film metal oxides have shown great character in gas sensing and optoelectronics. The n-type semiconducting oxides (n-TCOs) such as tin oxide (SnO_2) [1], zinc oxide (ZnO) [2], cadmium oxide (CdO) [3], have shown their applicability in the field of gas sensing.

Several thin film deposition techniques viz: chemical bath deposition (CBD) [4], thermal oxidation [5], pulsed laser deposition (PLD) [6,7], successive ionic layer adsorption and reaction (SILAR) [8] spray pyrolysis (SP) [9-13] and advanced spray pyrolysis (ASP) etc.[14] have been developed by researchers to synthesize semiconducting metal oxide thin films. The spray pyrolysis (SP) is technique is one of the effective and low cost technique as it is simple and gives reproducibility of results as compared to other techniques [13]. The advanced spay pyrolysis (ASP) technique involves modification in spray mechanism due to addition of furnace in its design [15,16]. Here, metal oxide thin films are deposited on to a pre-heated substrate by atomizing very fine droplets of a precursor solution, usually in the form of salt of metal oxide. Compressed air or neutral gas forces the fine droplets of solution into core of furnace and then arrive at preheated substrates mounted on the top of furnace. The constituents of precursor undergo pyrolytic decomposition inside the core of furnace (reaction chamber) and undergo nucleation when they reach the hot substrates so as to form a desired metal oxide thin film on top of the substrate. Hence, core and substrate temperature are important parameters of ASP technique in thin film formation and they control the physical and chemical properties of the metal oxide films.

2.0 Experimental

A pre-optimized, 0.02 M aqueous solution of high purity (99.9%) cadmium acetate (Thomas Baker, India)

was prepared in double distilled water to obtain undoped CdO thin films. A 300ml chemical solution was atomized through a glass nozzle and the reactants were allowed to undergo thermal decomposition in the core of furnace (reaction chamber). The metal oxide particles were pushed towards glass substrates and were deposited on it. During the course of deposition, the nozzle to substrate distance was kept at 41 cm and compressed air at a pressure of 10.5 LPM (litre per min.) was maintained to control the solution spray rate at ~5 ml/min. Undoped CdO thin films were synthesized at optimized core and substrate temperature of 325°C & 210°C respectively.

Similarly, 0.02 M, 300 ml spray solution was prepared in doubled distilled water by mixing 0.02 M solutions of high purity cadmium acetate [Cd $(CH_3COO)_2$. $2H_2O$] (Thomas Baker, India) and zinc acetate [Zn $(CH_3COO)_2$. $2H_2O$] (Thomas Baker, India). The solutions were mixed in appropriate proportion so as to get 300 ml of spraying solution and were atomized through a glass nozzle at core temperature of 325°C and substrate temperature of 210°C. The reactants undergo pyrolytic decomposition inside the core of furnace to form oxide particles and are pushed on to the glass substrates tightly fitted to the substrate holder. The substrate holder was mounted on top of furnace. Initial deposition parameters were retained as above. To study the effect of percentage of mixing on $Cd_XZn_{1-X}O$ film properties, films were deposited onto glass slides kept at constant substrate temperature 210°C. Ho and Lee [17] reported that stable ZnO phase can be obtained from Zinc acetate in the temperature ranging from 180 to 315 °C ; hence, the core temperature where the actual decomposition occurs was optimized at constant at 325 °C. The films formed with the variation of x from x=1, 0.4 and 0 were designated as M_0, M_{60} and M_{100} respectively.

As synthesized undoped CdO and mixed oxide $Cd_XZn_{1-X}O$ films were characterized for XRD, UV-VIS spectroscopy, SEM and H_2S gas sensing studies. The structure and phase formation of the films was characterized using X-ray diffraction method [Analytical instruments, Pvt. Ltd. Germany] in the scanning range of 20–80° at a step size of 0.1°. The Cu K_α (λ = 1.5406 Å) radiation was used as incident radiation. Surface morphology of typical samples was studied by field emission scanning electron microscopy FESEM (Nova NanoSEM 200) and JEOL JSM-6360 scanning electron microscope (SEM). Transmittance and absorbance was measured by using UV-VIS-NIR spectrophotometer (made by Schimadzu, Japan) in the scanning range 300-900 nm. A homemade gas sensing unit was used to study gas sensing performance of H_2S gas.

3.0 Results and Discussion

3.1.1. X-ray diffraction analysis

XRD patterns of typical $Cd_XZn_{1-X}O$ thin films M_{60} and M_{100} are compared with undoped CdO sample M_0 is shown in Fig.1. From the figure, it is seen that the films deposited with variation of x are polycrystalline in nature. However, peak intensities are found to decrease with increase in zinc percentage in the film. For x=1, i.e. for pure CdO (Mo sample), the film exhibits polycrystalline nature having rock salt type crystal structure with preferential orientation along (111) and (200) planes and all the peaks match with the JCPDS card no 05-640 indicating the formation of pure CdO. For x=0, i.e. for pure ZnO (sample M_{100}) there is complete shift in the structure from rock salt to wurtzize and the peaks match with those given by JCPDS card no 05-664 indicating the formation of pure ZnO. For sample M_{60}, the peak positions resemble to those of CdO, in addition to this, two peaks near to CdO peaks are seen, the shift in position of the peaks with respect to those of CdO may be due to presence of ZnO. Such type of behavior is reported by other researchers [18-20].

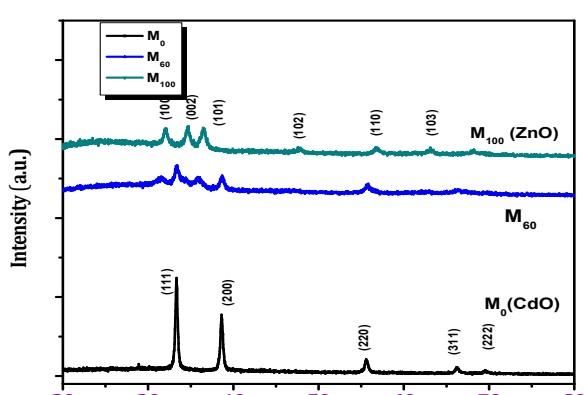

Fig.1: XRD patterns of undoped CdO and $Cd_XZn_{1-X}O$ thin films[16]

3.1.2. Surface morphological analysis

Fig.2. shows SEM and FESEM micrographs of sample M_0 and sample M_{60}. It is seen that the surface of the films is uniform and granular in nature however, for sample M_{60}, uniformly porous grains are seen with relatively less grain size (~54 nm) indicating that the sample is nanocrystalline with relatively large specific ratio which is requisite for exhibiting good gas sensing performance.

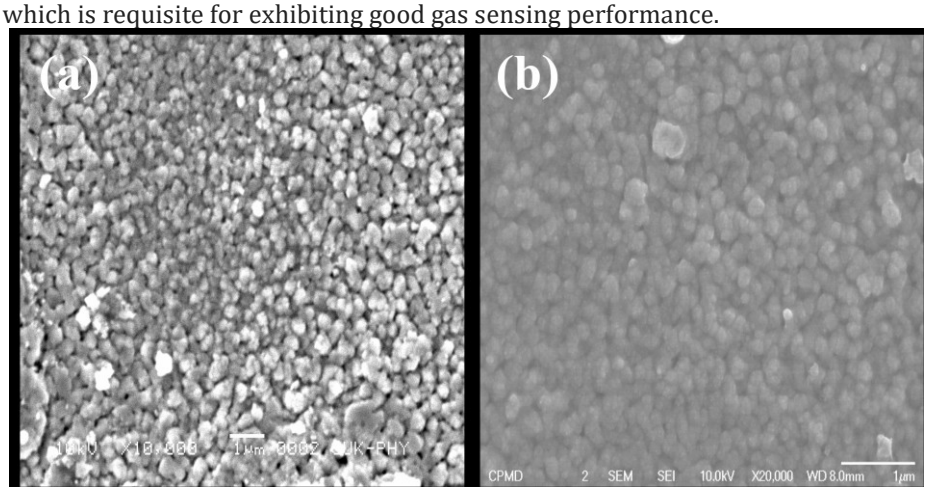

Fig. 2: SEM micrographs of samples(a) M_0 and(b) M_{60}

3.1.3: Optical analysis

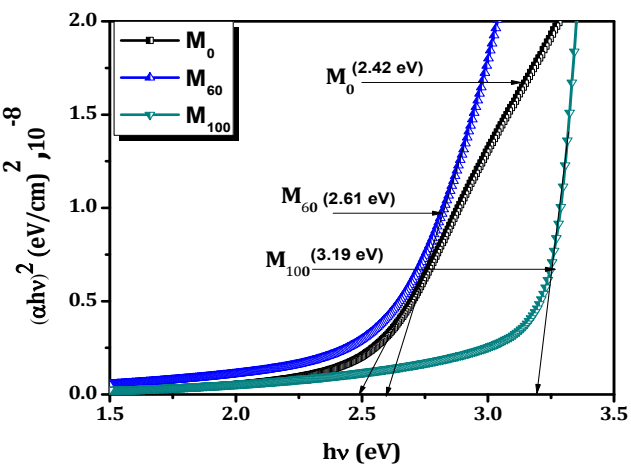

Fig.3: Plot of $(\alpha h\nu)^2$ vs. $(h\nu)$ for the samples M_0, M_{60}, M_{100} [14]

The optical absorption data was further analyzed to determine the band gap of typical samples. To obtain band gap, the graph of $(\alpha h\nu)^2$ versus hν was plotted for all samples as shown in Fig 3. The band gap energy 'E_g' is determined by extrapolating the linear portion of the curve to the x- axis [21]. The measured value of the band gap varies from 2.42 to 3.18 eV indicating that films are semiconducting in nature and band gap was found to increase with increase in zinc content.

3.1.4: Gas sensing analysis

Typical samples of undoped cadmium oxide (M_0) and cadmium zinc mixed oxide (M_{60}) thin films were subjected to known concentrations of H_2S gas at different operating temperatures. Fig.4. shows variation in sensitivity shown by sample M_0 of undoped cadmium oxide thin film at various concentrations of H_2S gas at operating temperature of 200°C, it is found that sensitivity increases with increase in concentration of H_2S gas.

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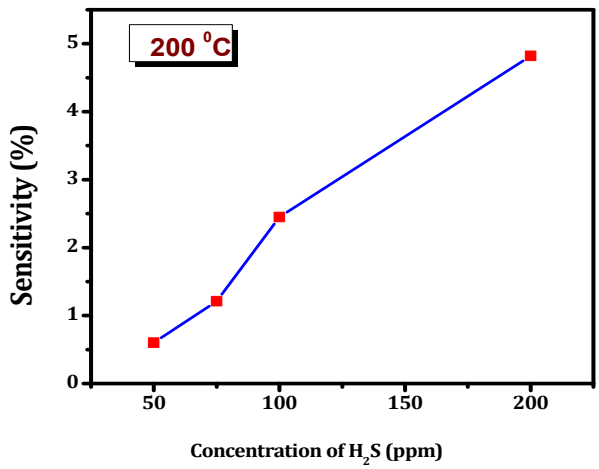

Fig.4: Plot of Sensitivity versus H_2S concentration at operating temperature 200°C shown by typical undoped CdO (sample M_0) thin film

The same sample was then subjected to different operating temperatures keeping the fixed concentration H_2S at 200 ppm. It was observed that, sensitivity increases with increase in temperature up to 250°C and then decreases at operating temperature 300°C as shown in Fig.5. This may be due to the fact that at low operating temperature, H_2S molecules do not have sufficient thermal energy to react with the adsorbed oxygen species at surface and film shows low sensitivity at low operating temperature [22]. At higher operating temperature the adsorbed oxygen gets converted from O_2^- to O^-. The gas sensing reactions that takes place at the surface reported by Shewale et al [23] are :

$$H_2S\ (g) + O_2^-\ (ads) \longrightarrow H_2\ (g) + SO_2\ (g) + e^-$$

$$H_2S\ (g) + 2O^-\ (ads) \longrightarrow H_2\ (g) + SO_2\ (g) + 2e^-$$

The increase in sensitivity at 250°C may be due to the supply of adequate thermal energy to overcome inter-granular barrier height. The decrease in response at higher temperature (300°C) may be due to the excess thermal energy that may slow down the process of interaction with oxygen.

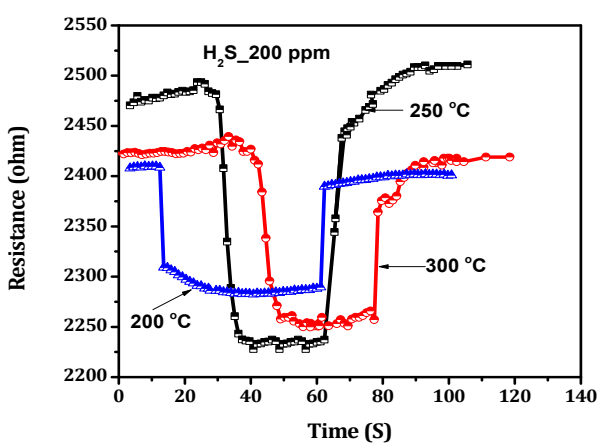

Fig.5: Resistance vs. time plots for undoped CdO thin film for H_2S gas concentrations of 200 ppm at different temperatures

Further, similar gas sensing measurements were carried out for a typical sample M_{60} of cadmium zinc mixed oxide thin film. Fig.6. shows variation in sensitivity at various concentrations of H_2S gas at operating temperature of 200°C.

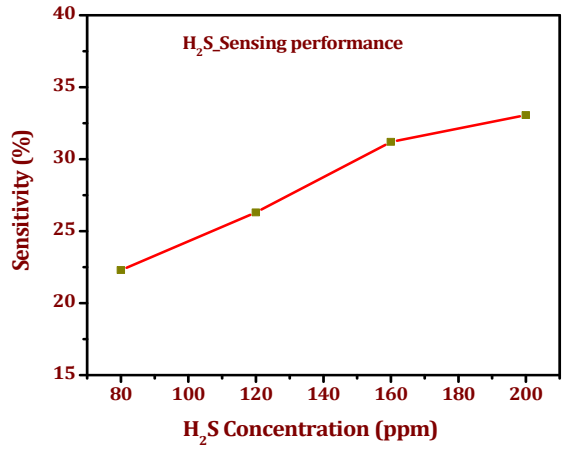

Fig.6: Plot of sensitivity versus H_2S concentration at operating temperature 200°C shown by typical undoped CdO (sample M_0 thin film

Moreover, it was observed that the sensitivity increases as H_2S concentration increases for both undoped and mixed oxide samples, however there was a remarkable increase in sensitivity shown by mixed oxide sample M_{60} as compared to undoped sample M_0 as shown in Fig.7. This clearly indicates that mixed oxide thin film is more sensitive than undoped film and also found to show better sensitivity towards relatively smaller concentrations of H_2S.

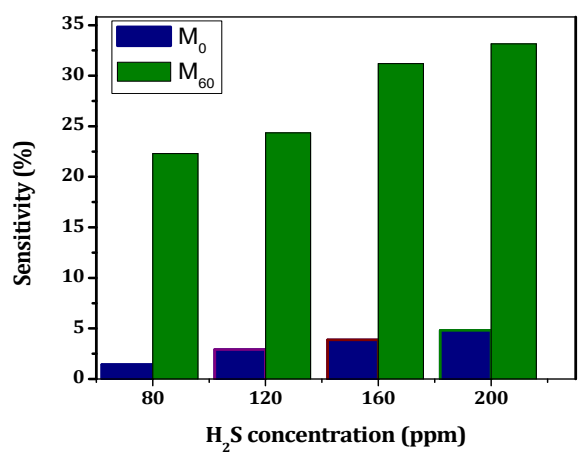

Fig.7: Bar chart showing comparison of sensitivity at various H_2S concentrations at operating temperature 200°C shown by typical samples M_0 and M_{60}

4.Conclusion

Undoped CdO and cadmium zinc mixed oxide thin films have been successfully deposited at low substrate temperature (210°C) with the help of novel advanced spray pyrolysis technique. The effect of zinc mixing on structural properties of was studied by XRD and SEM. XRD results show that films are polycrystalline in nature with cubic structure for undoped films. With increase in zinc content, there is shift in structure to wurtzize for pure zinc oxide sample. Similarly, from optical properties it is observed that the films are semiconducting in nature and shift in band gap was observed from 2.42 eV for undoped CdO to 3.18 eV for pure ZnO sample. SEM images reveal that the films were crack free and uniform having granular surface morphology. A comparative study on H_2S gas sensing performance indicates that cadmium zinc mixed oxide thin film was more sensitive at low gas concentrations than undoped cadmium oxide film which showed feeble sensitivity.

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Physico-electrochemical investigation of electrodeposited nanocrystalline Sb_2Te_3 thin films

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ABSTRACT: *In this study nanocrystallineantimony telluride (Sb2Te3) thin fims have been synthesized by potentiostatic mode of electrodeposition on stainless steel substrate. The electrochemical active surface area (ECSA) and structural properties are investigated. The effect of deposition time on physico-chemical and electrochemical properties also have been studied with various characterization techniques such as CV,XRD, FE-SEM, EDS, FT-Raman, wettability, XPS, TEM and EIS. The deposition potential -210mV was derived from CV analysis; this potential kept constant for further deposition. The deposited material have rhombohedral crystal structure with maximum crystallite size 35 nm and cauliflower like morphology. Maximum ECSA (2.77 cm²) is found at deposition time 400 second.*

Keywords: *nanocrystaline Sb2Te3; electrodeposition; potentiostatic; hydrophilic; ECSA; EIS.*

Highlights:

I. Synthesis of nanocrystaline Sb2Te3 by potentiostatic electrodeposition.
II. Investigation of structural, morphological and electrochemical properties of Sb2Te3 thin films.
III. Wettability study on deposited films.
IV. EIS analysis and ECSA calculation.

1. Introduction

Antimony Telluride (Sb2Te3) is V-VI group binary compound showing semiconductor behavior. [1]. The primitive cell of Sb_2Te_3 is rhombohedral structure and its conventional cell is hexagonal crystal structure similar to graphene [2] with a space group of R 3m (No.166) and the stoichiometric compound has the lattice parameters of a=4.271 Å, c=30.451 Å and c/a=7.130 [3].Lawal et al. reported that the Antimony Telluride is a low and direct band gap material, they theoretically calculated band gap equal to 0.221 eV [2].Sonawane et al. synthesized Sb2 Te3 thin films by electrodeposition; they observed the material is p-type semiconductor having band gap 0.3 eV [4].This chalcogenide material has verity of applications such as thermoelectric [5-9], phase-change memory [10] and low ohmic resistive contacts for CdS/CdTe film solar cell [11-21], thermal and humidity sensors using Seebeck and Peltier effects [22-26] etc. Liu et al.[27] studied the effects of substrate temperatures on to the structural, optical and electrical properties of Sb_2Te_3 films. They observed that increasing substrate temperature influence the growth kinetics of the films. Also, they reported that the absorption coefficient of Sb_2Te_3 film is above 10 Vcm^{-1} which is in the infrared range and corresponding optical band gap energy is around 0.32 eV. Lensch-Falk et al. [28] prepared polycrystalline and stoichiometric Sb_2Te_3 thin films by pulsed electrodeposition at room temperature. They reported that thermal conductivity of synthesized thin films varied with electrodeposition conditions and microstructure.

Sb2Te3 thin films were prepared by different physical and chemical methods such as, hydrothermal route [29-32], spin-coating method [33], co-evaporation [34, 35], molecular beam epitaxy [36, 37], RF-sputtered [38], Ion Beam Sputtering [39], and Electrodeposition [40]. Among these methods, the electrodeposition is prominent deposition method due to its several advantages such as easy to handling, flexibility, inexpensive, vacuum-less, suitable for large area, deposition is possible at room temperature and

reproducibility related to other methods. In this technique, films could be prepared by galvonostatic as well as potentiostatic method. The growth rate can easily be controlled through electrical quantities, such as current density and deposition potential.

In this article, electrodeposition method is employed for synthesis of polycrystalline Sb_2Te_3 films on stainless steel substrate with different deposition times ranging from 200 to 500 s. Sb_2Te_3 thin films have been produced without the use of surfactant into the electrolyte solution containing Sb^{3+} and Te^{2-} ions. Influences of different thicknesses onto structural, morphological, electrochemical and wettability properties of Sb_2Te_3 films are studied.

2. Experimental

2.1 Substrate cleaning

The electrically conducting substrates were used for deposition of thin film by electrodeposition method. The surface of the substrate provides nucleation sites for growth of thin films. The contaminated surface gives non-uniform growth onto the substrate. Therefore, substrate cleaning is an important part in the deposition of uniform thin films onto the conducting substrate by electrodeposition method. Usually, metallic substrates (copper, aluminium, stainless steel etc.) and conducting glass (FTO and ITO etc.) are used for electrodeposition method. In the present study the metallic substrates (Stainless steel) with dimensions 5 cm x 1 cm x 0.1 cm were used for deposition of Sb_2Te_3 films. Following processes were used for the cleaning of the substrates [41].

- Firstly substrates were mirror polished using zero grade polish paper.
- Polished substrates were washed with detergent powder and double distilled water. After that it was also rinsed in the double distilled water.
- The rinsed substrates were etched with 25% dilute HCl for 20 s and then cleaned ultrasonically.
- Finally, they were dried in vapors of alcohol.

2.2 Materials

Antimony chloride ($SbCl_3$) and sodium tellurite (Na_2TeO_3) as precursors were purchased from Alfa Aesar, Mumbai. Ethylene diamine tetra acetic acid (EDTA) (LobaChemie, Mumbai) was used as complexing agent. Tartaric acid(LobaChemie, Mumbai) and double distilled water were used as solvents. All chemicals were used without further purification.

2.3 Experimental setup

In electrodeposition, thin film is deposited on respective electrode of an electrodepotion cell comprising of working electrode (W), counter electrode (C), reference electrode (R) and electrolyte solution. The electrodepotion cell consist of following components.

a. Electrolyte
b. Electrodes
c. Power supply

The schematic diagram of experimental set up for electrodeposition by three electrode system is shown in fig.1.

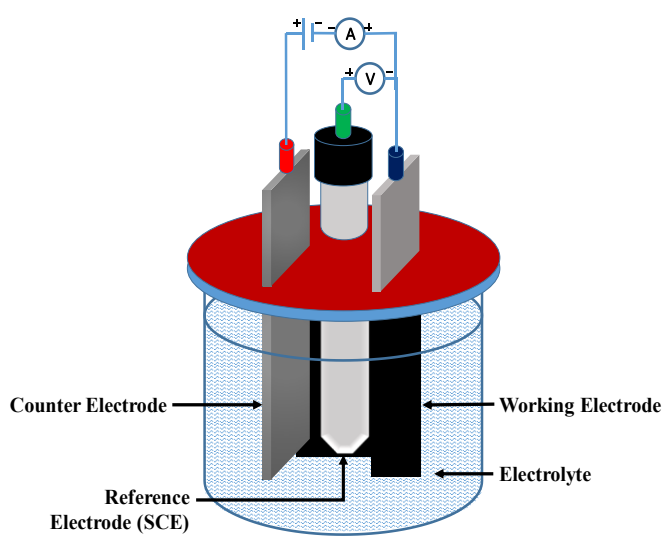


Figure 1: The schematic experimental setup for electrodeposition

2.4 Synthesis of Sb_2Te_3 thin films

Antimony telluride (Sb_2Te_3) thin films of different thicknesses were grown onto the stainless steel substrates using cost effective potentiostatic electrodeposition technique. All the depositions were taken at room temperature by using a three-electrode cell setup. For preparation of electrolyte solution containing Sb^{3+} and Te^{2-} ions, 0.04 M antimony chloride was dissolved in 40 ml 0.2 M tartaric acid and 0.04 M sodium telluride was dissolved in 60 ml double distilled water separately. These two solutions mixed together and 0.04 M 10 ml EDTA solution was added dropwise in it. The final volumetric proportion of Sb:Te:EDTA was 4:6:1. Further the prepared electrolyte solutions (containing $Sb^{3+}$and Te^{2-} ions) were used to synthesis of uniform Sb_2Te_3 thin films by electrodeposition method. The volumetric ratio was kept constant for all electrodeposition experiments. The Sb_2Te_3 films were prepared with different deposition time ranging from 200 s to 500 s at interval of 100 s. The detailed preparative parameters for preparation of Sb_2Te_3 thin films by electrodeposition methods are summarized in Table 1. Synthesized thin films of Sb_2Te_3 by electrodeposition method were abbreviated as ST_2, ST_3, ST_4 and ST_5 for deposition times 200 s, 300 s, 400 s and 500 s respectively for a same volumetric ratio of Sb:Te:EDTA in electrolyte solution.

Table 1: Preparative parameters of electrodeposition method for preparation of Sb_2Te_3 thin films.

Sr. No.	Parameter	Values
1	Medium	Non-aqueous
2	Total quantity	25 ml
3	Bath composition	0.04 M $SbCl_3$ + 0.04 M Na_2TeO_3
4	Concentration of EDTA	0.04 M
5	Applied potential	-0.260 V/SCE
6	Deposition temperature	297 K
7	Substrate	Stainless steel
8	pH	~ 1.8

2.5 Characterization of Sb_2Te_3 thin films

Electrodeposited Sb_2Te_3 thin films were characterized by various characterization techniques such as structural, morphological, elemental, wettability and electrochemical properties as discussedbelow,

X-ray diffraction (XRD) patterns of Sb_2Te_3 films were recorded by Bruker powder X-ray diffractometer, Model D2: phaser with CuK_α radiation of wavelength 1.5406 Å. Identification of phase and crystalline nature of Sb_2Te_3 thin films were analyzed from XRD patterns using X' Pert high score plus software. Fourier transform Raman (FT-Raman) spectrum was recorded using Bruker MultiRAM, Germany over the range of 200-600 cm^{-1} excited with Nd:YAG laser source (excitation wavelength was 1064 nm). Thicknesses of synthesized Sb_2Te_3 films were calculated by using weight difference method (ρ=M/V g/cm^3). X-ray photoelectron spectroscopy (XPS) study gives the information about the chemical composition, valence state of elements and concentration of elements on the surface of films. A monochromatic X-ray beam (Energy = 1253.6 eV for Al Kα) were used in XPS study. XPS peaks were calibrated with binding energy of C 1s peak located at 284.78 eV. Survey and narrow scan XPS spectra of Sb_2Te_3 film were recorded at energy step size of 1.000 and 0.100 eV respectively. The surface morphology of synthesized Sb_2Te_3 films were studied using field emission scanning electron microscopy (FE-SEM), (Model: MIRA 3 LMH). Elemental analysis of Sb_2Te_3 film was carried out using EDS techniques attached with FE-SEM techniques (NCA x-Act detector of Oxford instruments, USA). The detailed information of microstructure, particle size and elemental composition on the surface of Sb_2Te_3 film was obtained using field emission transmission electron microscopy (FE-TEM) technique. The interaction between liquid and surface of Sb_2Te_3 thin films were studied by measuring water contact angle between them using Rame-hart USA equipment with CCD camera. Electrochemical impedance spectroscopy (EIS) analysis of Sb_2Te_3 films was carried out using electrochemical workstation (AUT85804, Netherlands, frequency range from 10^6 Hz to10^{-6} Hz in AC mode (100 mV).

3. Results and discussion

3.1 Film formation and reaction mechanism

Cyclic voltammogram studies were carried out to determine the appropriate deposition potentials for an electrosynthesis of Sb_2Te_3 thin films. The potentials are swept over a range from -1.0 to +1.0 V at a

scan rate 20 mv/S. Fig. 2 (a) shows the CV of unitary Sb whose concentration is 0.04 M $SbCl_3$ in electrolyte solution. It shows the presence of one oxidation peak at 0.057 V/SCE and one cathodic peak at -0.52 V/SCE which corresponds to the reductive deposition potential of Sb^{3+} in electrolyte solution. Fig. 2 (b) shows CV of Te system (0.04 M sodium tellurite in electrolyte solution). It also shows one oxidation and reduction peak at 0.611 V/SCE and -0.109 V/SCE respectively. The reduction peak at -0.109 V/SCE represents the consecutive reduction of $HTeO_2^+$ to Te^{2-} in electrolyte solution [42]. Fig. 2 (c) shows cyclic voltammograms (CVs) of binary equimolar Sb and Te solutions deposited onto the stainless steel substrate.

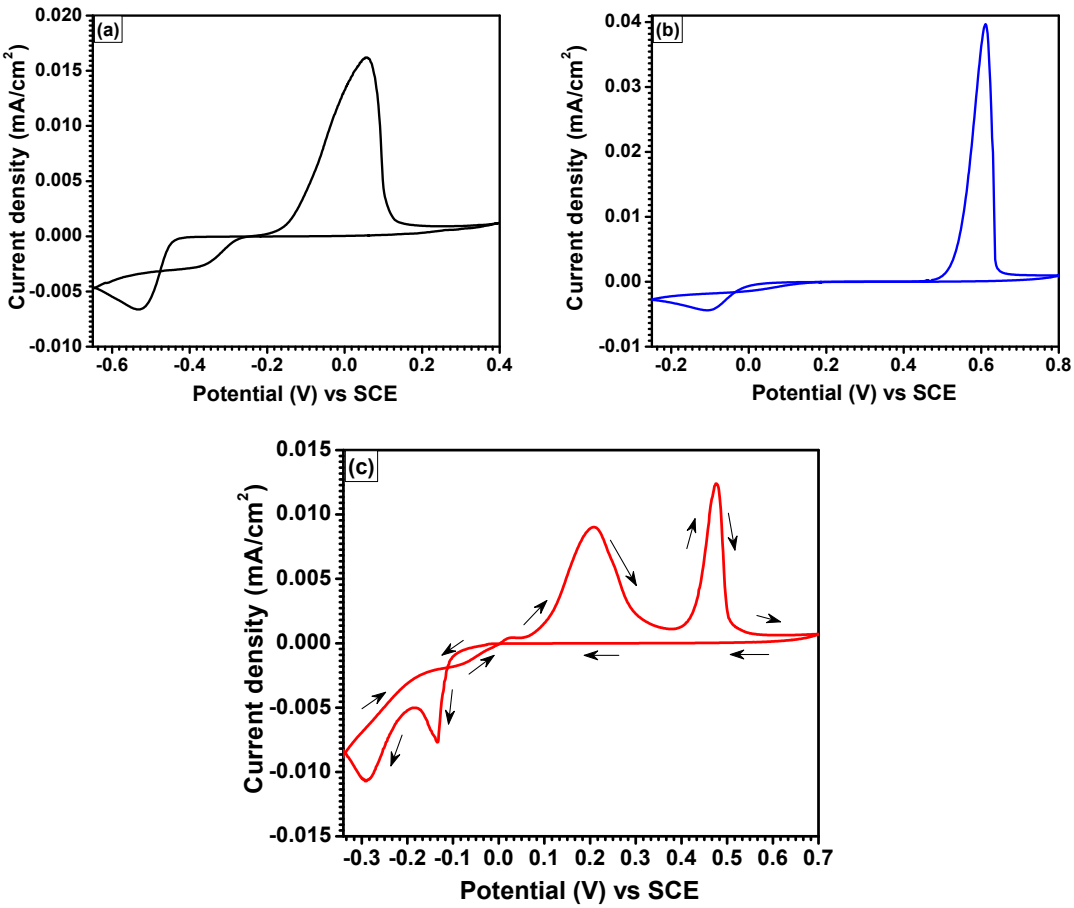


Figure 2: Cyclic voltammograms profiles of (a) unitary Sb (0.04 M), (b) unitary Te (0.04 M) and (c) mixed Sb (0.04 M) and Te (0.04 M) system

CV of equimolar Sb and Te solution shows two anodic peaks situated at 0.20 V/SCE and 0.49 V/SCE respectively while two cathodic peaks approximately at -0.125 V/SCE and -0.294 V/SCE respectively. The first main reduction peak located at -0.125 V/SCE, which correlates to theformation of SbTe on subsrate whereas the following reduction reaction occurs at -0.294 V/SCE because of hydrogen evolution[7, 43]. The two oxidation peaks appears in CV corresponds to the process of stripping the Te atoms. Therefore, a deposition potential range between -0.125 V/SCE and -0.294 V/SCE to be an appropriate for electrodeposition of SbTe.The reaction taking place during the deposition processis given as [43].

$$3HTeO^{2+} + 18e^- + 2SbO^+ + 13HTeO^{2+} \leftrightarrow Sb2Te_3 + 8H_2O.$$

The suitable deposition potential to obtain more nearly stoichiometric composition (40 atomic % Sb and 60 atomic % Te) of Sb and Te in Sb_2Te_3 is to be determined from dependence between deposition potential and atomic composition in CV potential range and its value is -0.260 V/SCE.So Sb_2Te_3 thin films were deposited at -0.260 V/SCE. During deposition current density initially increases with respect to time and further remains constant up to the time 450 s. Above 450 s, current density slightly decreasedcathodically; it is attributed to beginning of overgrowth of the film.The actual photograph of Sb_2Te_3 thin films deposited on stainless steel substrate with deposition time varies from 200 s to 500 s in steps 100 s at deposition potential -0.260 V/SCE are shown in fig. 3.

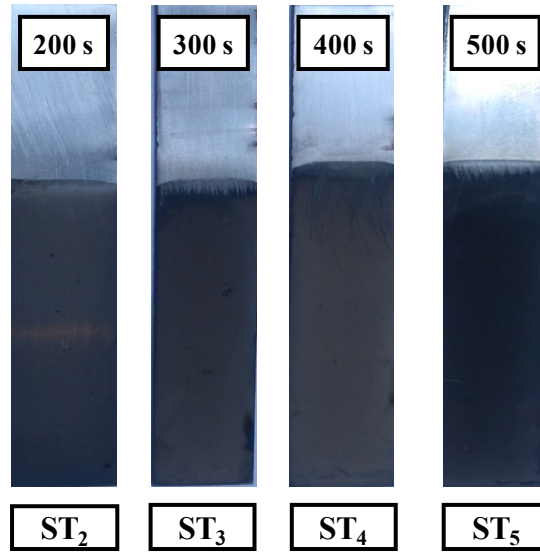


Figure 3: Photographs of electrodeposited Sb_2Te_3 thin films with various deposition times

3.2 X-ray diffraction (XRD) studies

Fig. 4 shows the XRD patterns of Sb_2Te_3 thin films grown at different deposition times. It apparently shows the formation of highly crystalline Sb_2Te_3 thin films.

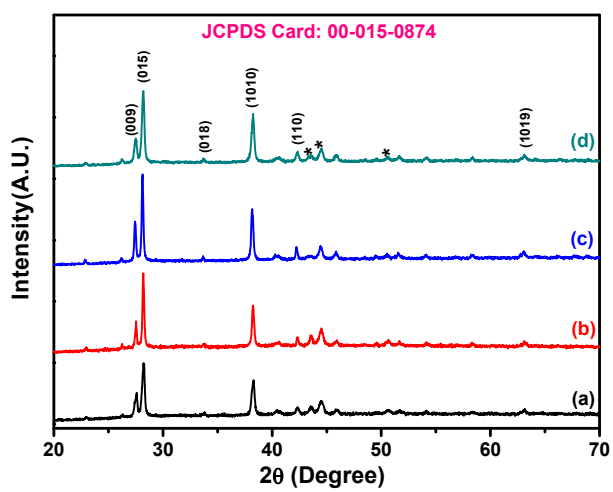


Figure 4: XRD patterns of **(a)** ST_2, **(b)** ST_3, **(c)** ST_4, and **(d)** ST_5, films deposited by electrodeposition

All diffraction peaks (excluding stainless steel peaks) are well matched with standard JCPDS card No. 00-015-0874. The indexed peaks of Sb_2Te_3 show the rhombohedral crystal structure of Sb_2Te_3 films. In addition to diffraction peaks of Sb_2Te_3, the one more peak is clearly visible at 28.5^0 in XRD patterns that represents peak of Te phase. The similar behavior is observed by J. M. Lin et al. for preparation of Sb_2Te_3 films by Thermal Evaporation method [44]. The peaks indicated with an asterisk (*) in XRD pattern represents peaks of stainless-steel. The diffraction peak corresponds to 37.5^0 represents the growth direction along (10$\underline{1}$0) plane. The crystallinity increases with increasing deposition time up to the 400 s and after that it decreases for higher deposition time. Crystallite size of synthesized Sb_2Te_3 films were calculated using Scherrer's formula along most intense plane [45]. The comparatively higher value of crystallite size is obtained for ST_4 film. Calculated values of crystallite size of Sb_2Te_3 films are summarized in Table 2. The average values of microstrain and dislocation density of Sb_2Te_3 thin films are calculated using equation (1) and (2).

$$\varepsilon = \frac{\beta \cos(\theta)}{4} \tag{1}$$

$$\delta = \frac{1}{D^2} \quad (2)$$

The values of microstrain and dislocation density of synthesized Sb_2Te_3 thin films are noted in Table 2. Thickness of Sb_2Te_3 thin films were calculated by using weight difference method [46].

Table 2: Crystallite size, microstrain, dislocation density and thicknesses of Sb_2Te_3 films.

Sample Code	Crystallite size (*D*) (nm)	Microstrain (ε), 1× 10^{-3}	Dislocation density (δ),1× 10^{-3}, lines m^{-2}	Thickness (*t*) (nm)
ST_2	23	1.8490	3.7720	584
ST_3	26	1.5190	2.1090	829
ST_4	35	1.2230	1.4480	1102
ST_5	33	1.2400	1.5370	1425

3.3 FT-Raman studies

Fig. 5 shows typical Raman spectrum of synthesized Sb_2Te_3 thin film deposited at 400 s. The five Raman bands are observed in Raman spectrum. Raman peaks corresponding to ~112 $cm^{-1}$and ~166 cm^{-1} are assigned to E_g^2 and A_{1g}^2 modes respectively [47]. The peaks located at ~36 cm^{-1} and ~72 cm^{-1} are ascribed to E_g^1 and A_{1g}^1 modes respectively [48]. Obtained results of Sb_2Te_3 film are consistent with literature data [34, 49]. From FT-Raman spectroscopy analysis, formation of Sb_2Te_3 thin films on stainless steel substrate has confirmed.

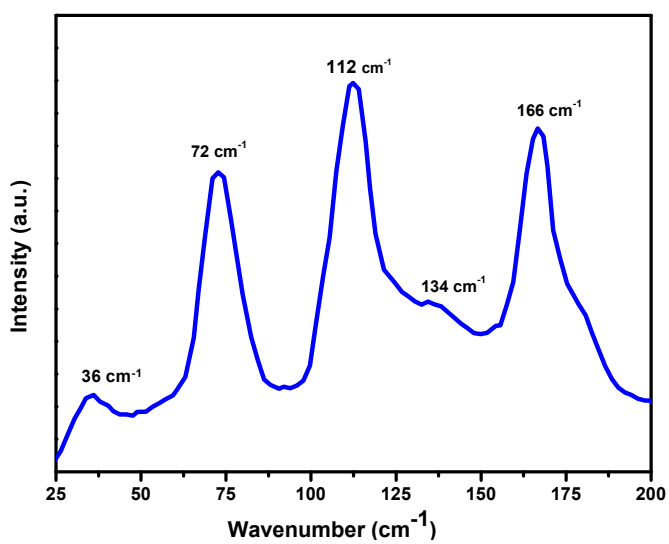


Figure 5: FT-Raman spectrum of ST_4 thin film

3.4 X-ray photoelectron spectroscopy (XPS) analysis

XPS analysis were carried out for identification of elements, their valance states and stoichiometry of Sb_2Te_3 film. Survey and narrow scans XPS spectra were recorded separately for ST_4 film. Fig. 6 (a) shows survey scan XPS spectrum of synthesized Sb_2Te_3 film. It shows various peaks associated with the core levels of Sb, Te and C. The observed Sb/Te ratio is 0.66 for ST_4 film. Binding energy values of core-level for Sb and Te are in good agreement with sub-stoichiometric Sb_2Te_3. Therefore, the electrodeposited Sb_2Te_3 film is sub stoichiometric. The Sb/Te ratio is more consistent with result analyzed from EDS analysis. Fig. 6 (b) shows the narrow scan XPS spectra of 3d core levels of Sb. The relative peak positions of Sb element are determined the chemical state of Sb element. Sb 3d peaks are fitted at 530.30 eV and 539.67 eV for Sb $3d_{5/2}$ and Sb $3d_{3/2}$ core levels respectively. The binding energy difference between Sb $3d_{3/2}$ and Sb $3d_{5/2}$ for 3d doublet of Sb is 9.4 eV, which is in good agreement with reported literature values for Sb_2Te_3 films [50].Fig. 6 (c) shows the narrow scan XPS spectrum of 3d core level of Te element. The XPS peaks at Te $3d_{5/2}$ and Te $3d_{3/2}$ corresponding values of binding energy are 572.99 eV and 583.37 eV respectively. XPS Peaks at 576.58 eV and 586.70 eV in Fig. 6 (c) are correspond to Te $3d_{5/2}$ and Te $3d_{3/2}$ core levels in Te 3d which indicates

that the existence of oxidized states of Te atom on the oxidized surface. Obtained results are well agreement with reported values of binding energies for Sb_2Te_3 film [51, 52].

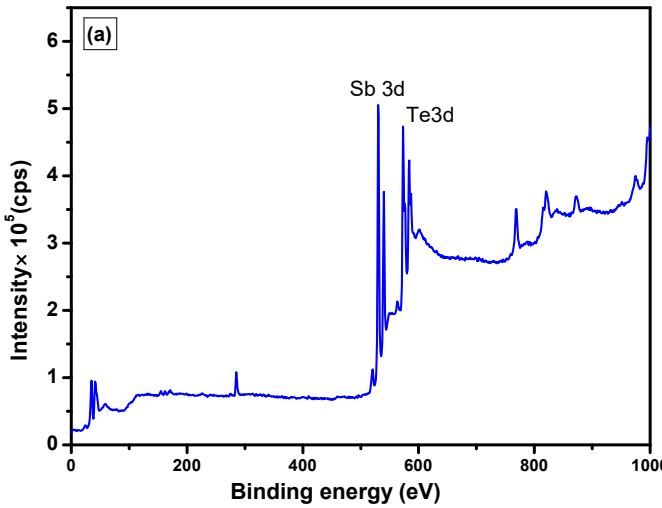


Figure 6: (a) Survey scan XP spectrum of the Sb_2Te_3 thin film deposited with 400 s

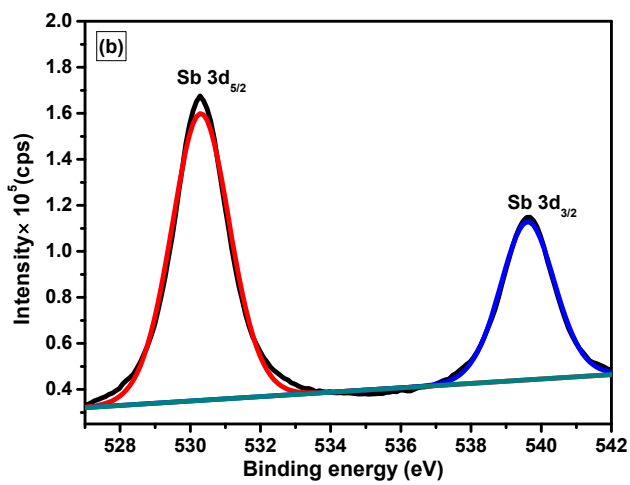


Figure 6: (b) Narrow scan XP spectrum of the 3d core level of Sb of the Sb_2Te_3 thin film deposited with 400 s

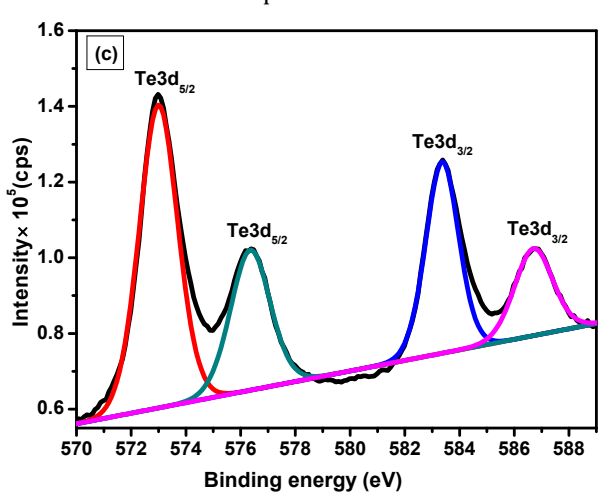
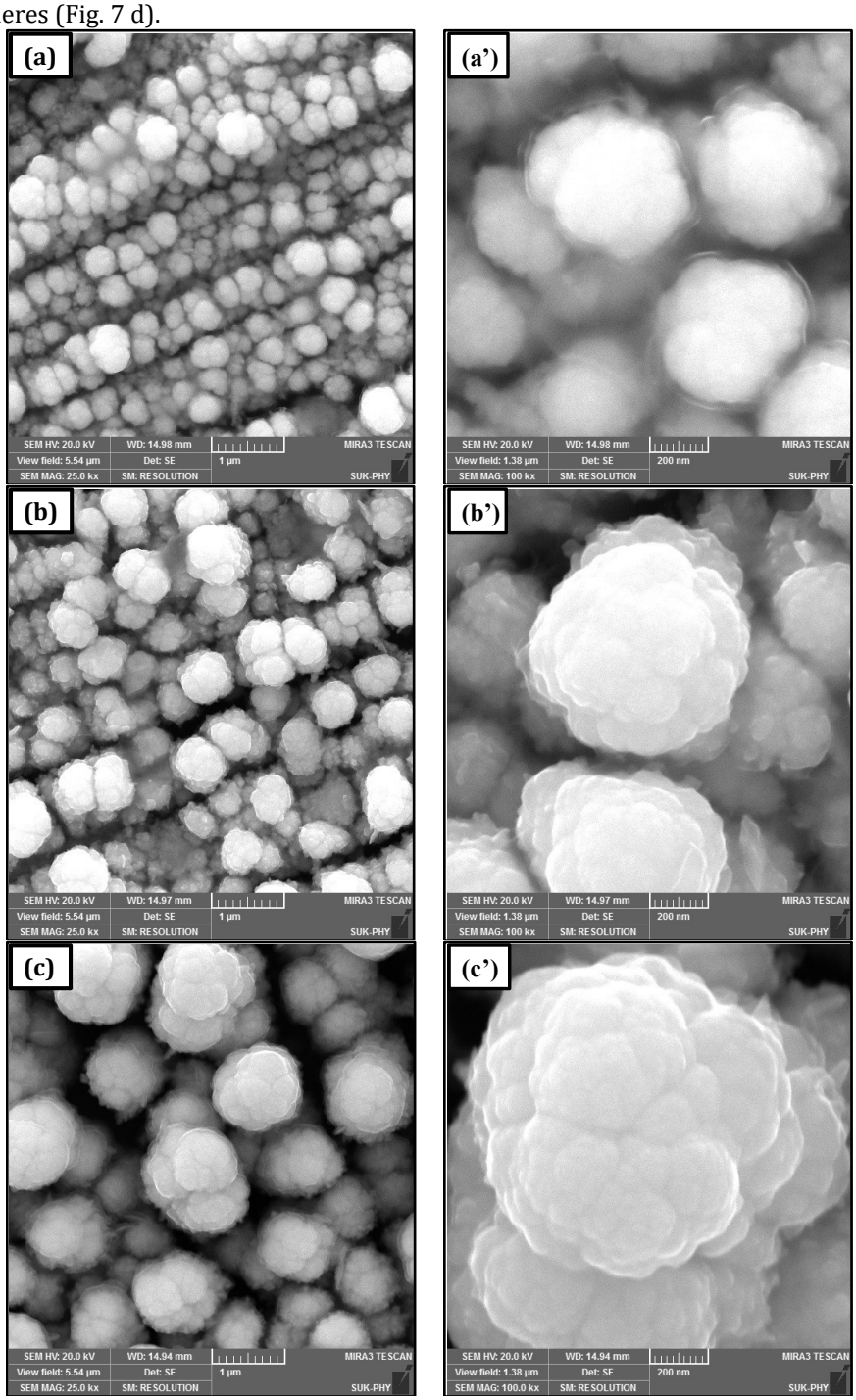


Figure 6: (c) Narrow scan XP spectrum of 3d core level of Te of the Sb_2Te_3 thin film deposited with 400 s.

3.5 Surface morphological study

Fig. 7 shows FE-SEM images of Sb_2Te_3 films deposited with different deposition times. It indicates existence of cauliflower like particles of Sb_2Te_3. The cauliflower like microstructure are uniformly distributed onto the surface of films. The average particles sizes of Sb_2Te_3 microsphere is approx. 985 nm for ST_4 film (Fig. 7 c). The average particle size increases with increasing deposition time. This may be due to the agglomeration of Sb_2Te_3 microspheres with increasing deposition time. As a result the formation of larger microspheres (Fig. 7 d).



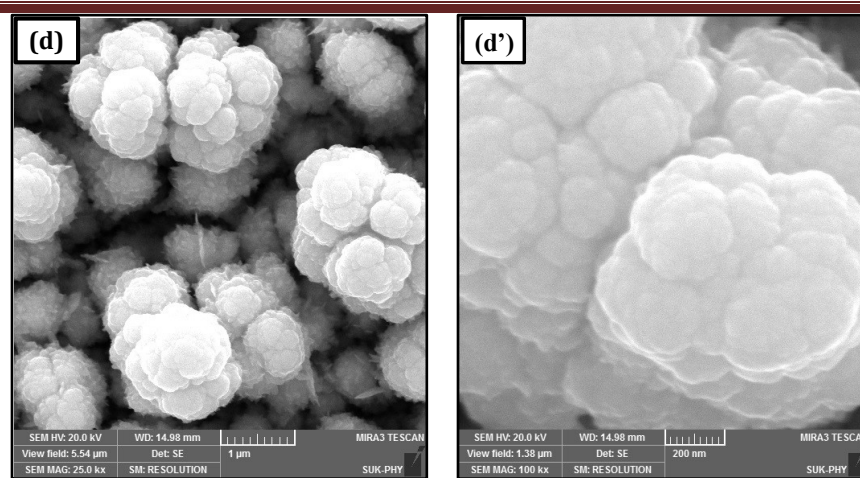


Figure 7: SEM images of **(a)** ST_2, **(b)** ST_3, **(c)** ST_4, and **(d)** ST_5films for 25 kX magnification and **(a')** ST_2, **(b')** ST_3, **(c')** ST_4, and **(d')** ST_5 films for 100 kX magnification.

The higher magnification (Fig. 7 a' to d') images of Sb_2Te_3 films are also show cauliflower morphology.

3.6 Energy dispersive X-ray analysis (EDS)

Fig. 8 shows a typical EDS spectrum of ST_4 film. Characteristic peaks of X-ray emissions corresponding to Sb and Te are clearly visible in EDS spectrum. The elemental composition of Sb_2Te_3 is nearly stoichiometric (Sb/Te is 0.594).

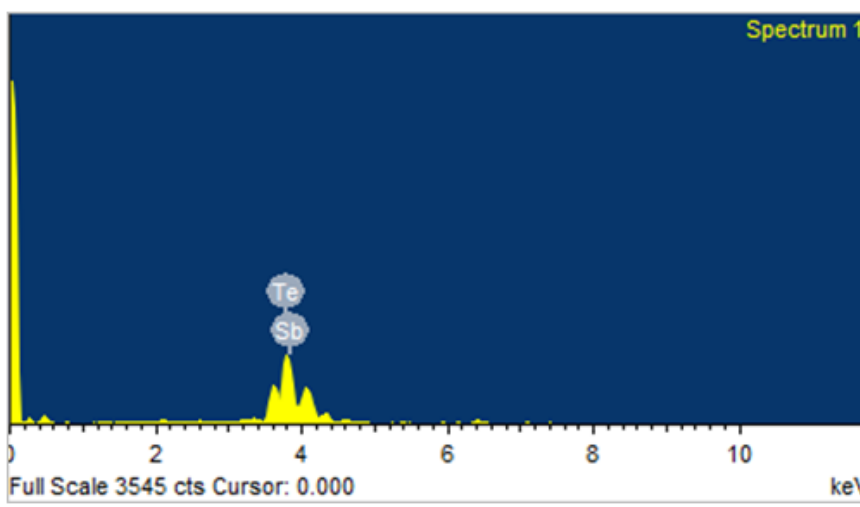


Figure 8: The typical EDS spectrum of ST_4film deposited at 400 s.

Table 3: Atomic percentage of typical ST_4 film measured using EDS spectrum.

Elements	Atomic %
Sb M	**37.28**
Te L	**62.72**
Total	**100**

3.7 Field Emission Transmission Electron Microscopy (FE-TEM)

FE-TEM characterization gives the additional information about the interior structure of Sb_2Te_3 microspheres. Fig. 9 shows (a) FE-TEM image and (b) High-angle annular dark field (HAADF) image of typical Sb_2Te_3 film deposited at 400 s. Fig. 9 (c-e) shows HAADF images for elemental mapping on surface of Sb_2Te_3 film to the corresponding area showing the presence of Sb and Te elements respectively. From Fig. 9 (d) and (e), it is clearly seen that the Sb and Te elements are uniformly distributed on the surface of synthesized Sb_2Te_3 film. The size of Sb_2Te_3 particles is about 904 nm which is consistent with results analyzed form FE-SEM.

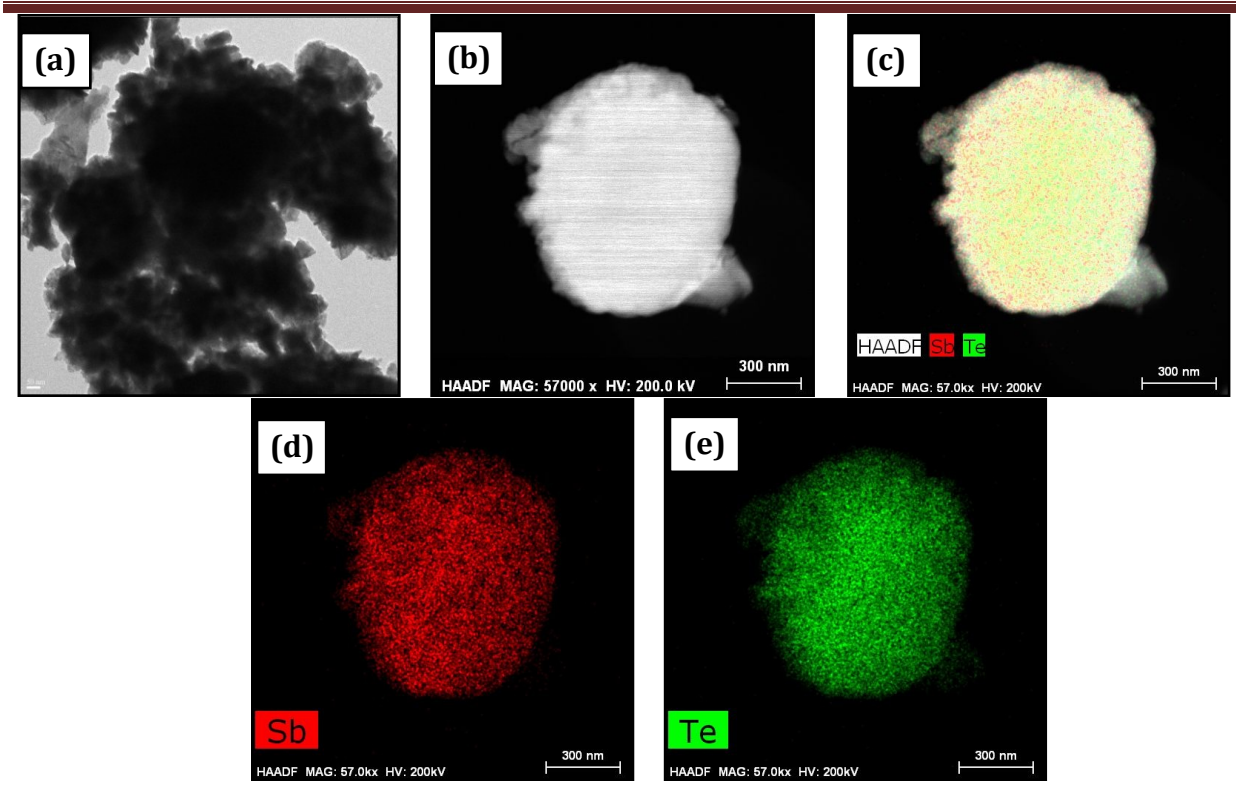


Figure 9: (a) FE-TEM image, (b-e) High-angle annular dark field (HAADF) images of typical Sb_2Te_3 film

3.8 Wettability study

Fig. 10 illustrates the variation of water contact angle (CA) of Sb_2Te_3 films with deposition time and inset shows the image of water contact angle of ST_4 film. Sb_2Te_3 films are found to be hydrophilic in nature. Water contact angle of prepared films increases with increasing deposition time upto the 400 s and decreases for higher deposition time. The variation in contact angle depends on the surface properties of the films. The maximum value of water contact angle is 75^0 for ST_4 film.

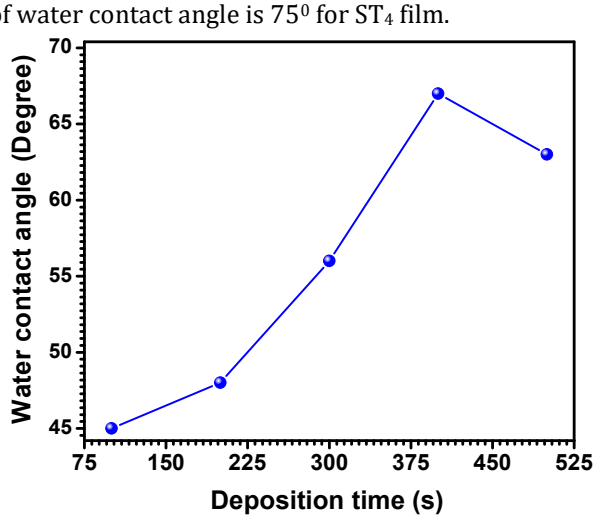


Figure 10: Variation of water contact angle with deposition time for Sb_2Te_3 films.

3.9 Electrochemical impedance spectroscopy analysis

Fig. 11 shows the Nyquist plots of synthesized Sb_2Te_3 thin films. Nyquist plots are recorded with cell configuration SS/Sb_2Te_3/1M Na_2HSO_4/graphite and at open circuit potential (OCP) 180 mV/SCE. It consists of two semicircles for each plots. The one semicircles in middle frequency region and other semicircles in high frequency region. The broad view of semicircles in high frequency region which is shown in inset of Fig. 11. The intersection of semicircle on Z_{real} axis in high frequency region gives the solution

resistance (R_s) of electrolyte, approximately it is same for all samples while its diameter gives the charge transfer resistance (R_1) of electrolyte, it is very small so these semicircles merge into next semicircles in middle frequency region. The diameter of semicircle for ST_4 film is smaller than that of other deposited film.Also, the values of charge transfer resistance decreases with increasing deposition time up to the ST_4 film and their after increases for higher deposition time. The obtained data for optimized ST_4 film from Nyquist curves are well fitted with equivalent electronic circuit (Fig.12) to evaluate the electrolyte solution resistance (R_s), charge transfer resistance (R_1), double-layer capacitance (C_1) at the electrode/electrolyte interface, recombination charge transfer resistance (R_2) and chemical capacitance (C_2) [53]. The values of fitting parameters are summarized in Table 4.

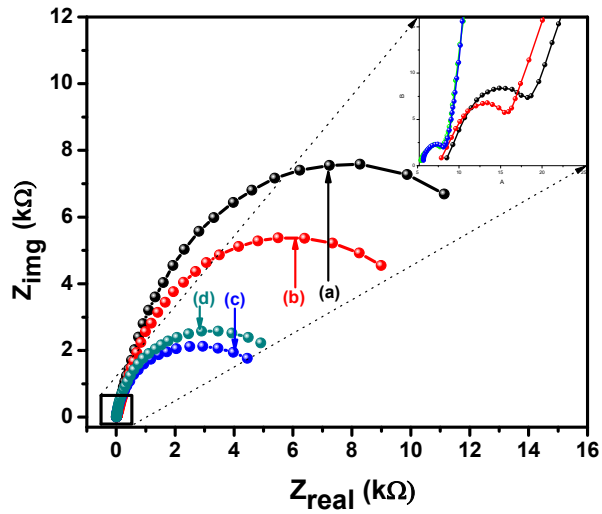


Figure 11: Nyquist plots for **(a)** ST_2, **(b)** ST_3, **(c)** ST_4, and **(d)** ST_5 films; (SS/ Sb_2Te_3/1 M Na_2HSO_4/graphite cell)

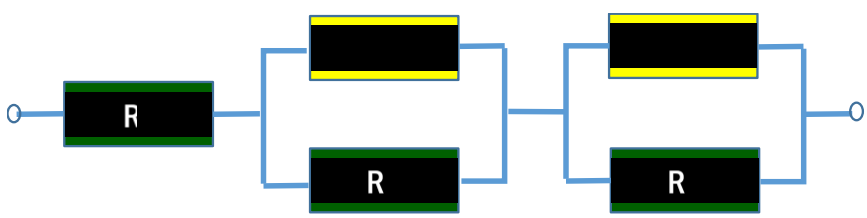


Figure 12: Equivalent circuit model for ST_4 film to analyze electrochemical impedance parameters

Table 4: EIS parameters for optimized ST_4 film.

EIS parameters	**ST_4**
R_s (Ω)	5.312
R_1 (Ω)	9.203
C_1 (F)	6.16×10^{-16}
R_2 (Ω)	6185
C_2 (F)	1.05×10^{-04}

Fig. 13 shows Bode plots of Sb_2Te_3 thin films deposited with various deposition times measured at open circuit potentials. From plots it is seen that the phase angle changes within 81.32 to 78.37^0. The phase angle decreases with increasing deposition time up to 78.37^o for ST_4 sample. After that phase angle increases for higher deposition time.

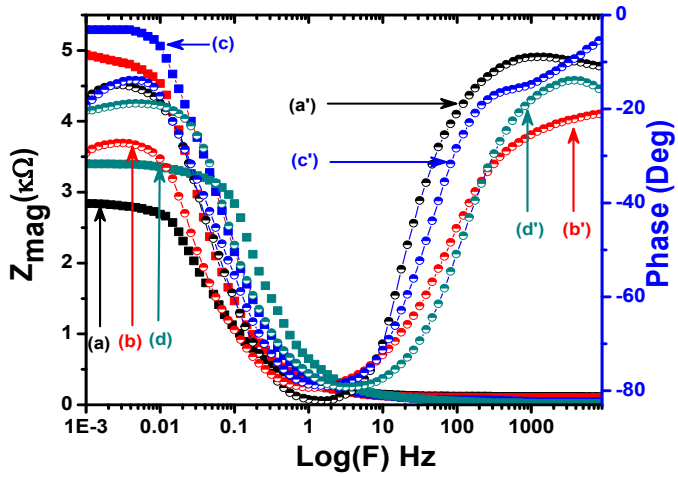


Figure 13: Bode plot for (a and a') ST_2, (b and b') ST_3, (c and c') ST_4, and (d and d') ST_5 films

In electrochemical studies the real surface area of films are electrochemically active surface area (ECSA) under working conditions. It gives the reactive surface sites of electrodes. The geometrical surface area of films impels that the higher surface area. But, these electrodes do not satisfy the conditions for smooth surface having low roughness values because high surface area comprise on low geometrical surface area of films. EIS technique was used for estimation of ECSA of films. ECSA of Sb_2Te_3 films are calculated from Bode curves which are measured under light radiation (frequency range from 10^{-1} Hz to 10^5 Hz at open circuit potential). The capacitances of films were estimated at frequency of 10 Hz according to following equation (3) [54].

$$C = \frac{1}{(2\pi f . Z_{10Hz})} \quad (3)$$

Where, Z is impedance corresponds to frequency at 10 H_z. The ECSA is ratio of estimated capacitance value (C) and standard capacitance value (C_0 = 45μf/cm²) for per square centimeter area of electrode. ECSA and impedance at 10 Hz of Sb_2Te_3 thin films are summarized in Table 5.

Table 5: Electrochemical active surface area (ECSA) and impedance at 10 Hz (of $Z_{(at\ 10Hz)}$ ST_2, ST_3, ST_4 and ST_5 thin films.

Sample Code	Phase Angle (deg)	$Z_{(at\ 10Hz)}$ Ω	ECSA cm²
ST-2	81.32	137.58	2.57
ST-3	79.32	133.21	2.66
ST-4	78.87	127.82	2.77
ST-5	79.19	135.81	2.61

Calculated ECSA shows the activities of films in term of real surface area that gives the reactive surface sites of films. The estimated values of ECSA are higher than the values obtained from geometric surface area (1 cm²). The ST_4 film shows higher ECSA (2.77 cm²) as compared to other films.

4. Summary and conclusions

The Sb_2Te_3 thin films have been deposited by electrodeposition methods. XRD analysis confirms that Sb_2Te_3 thin films have rhombohedral crystal structure. From FT-Raman spectroscopy analysis formation of Sb_2Te_3 thin films have been confirmed. XPS study indicates the presence of Sb and Te elements in different chemical states in Sb_2Te_3 films. Surface morphological study shows that deposited films exhibit cauliflower like morphology. Sb_2Te_3 thin films shows hydrophilic nature. The estimated values of ECSA are higher than the values obtained from geometric surface area (1 cm²). The ST_4 film shows higher ECSA (2.77 cm²) as compared to other films.

Acknowledgements

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GROWTH OF CARBON NANOTUBES FOR THEIR USE IN DYE-SENSITIZED SOLAR CELL

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ABSTRACT: *Multiwalled carbon nanotubes (MWCNTs) have been prepared via chemical vapor deposition (CVD) method. The influence of temperature on the evolution of MWCNTs and their use in the thin film ZnO-based dye-sensitized solar cell (DSSC) as a counter electrode has been reported. The ZnO photoelectrode has been deposited by ultrasound assisted modified successive ionic layer adsorption and reaction (M-SILAR) method. XRD and RAMAN analysis reveals the formation of hexagonal graphite structure of MWCNTs and hexagonal wurtzite crystal structure of ZnO. SEM study illustrates that, at 900 ^{0}C, MWCNTs are well developed and their diameter increases with increase in pyrolyzing temperature. Photoelectrochemical characteristics of DSSCs with a Pt-coated FTO (Pt: FTO) and MWCNTs as counter electrode have been also compared. DSSC prepared using ZnO-Pt: FTO showed the power conversion efficiency (PCE) of 0.56%, however, ZnO-CNT based DSSC exhibited somewhat less PCE of 0.42 %.*

Keywords: *Nanotubes; Chemical vapor deposition; Surface characterization; Microstructure; Electrochemical.*

1. Introduction

In the DSSC assembly, the wide band gap semiconducting material onto which dye molecules get adsorbed, has a substantial influence on the power conversion efficiency (PCE) [1]. Earlier reports proposed various photoactive materials for the DSSC anode, such as TiO_2, ZnO, ZrO_2, SnO_2 and Nb_2O_5 [2-6]. Now, ZnO is considered as a potential photovoltaic material to TiO_2 in DSSCs [7]. Counter electrode (CE) is another component which strongly affects the PEC. Platinum (Pt)-based CEs showed efficient performance because of their high catalytic activity and better conductivity [8]. But, Pt belongs to the noble metal group and the deposition methods need a vacuum facility, which limits the commercial success of DSSCs [9]. Therefore a research is going on to explore a new economic substitute material to replace the Pt -based CEs.

Recently, carbon materials such as carbon black, hard carbon spheres, graphite, carbon nanotubes (CNTs) and graphene have been used in DSSCs as CEs due to their high conductivity and low cost [10]. Among these, CNTs have been one of the most attractive objects due to their outstanding mechanical, thermal and electrical properties,which come from their unique carbon structures [11, 12]. Many synthesis methods like arc-discharge, laser ablation, chemical vapor deposition (CVD), plasma-enhanced CVD (PECVD) etc. are extensively used for CNT production [13-16]. As compared to above-stated methods, CVD is a simple and economic method for producing CNTs at low temperature and ambient pressure along with high yield and purity[17]. Zeng et al. synthesized the ZnO-coated vertically aligned CNTs onto a stainless steel substrates by using N719 sensitizer with varying dye loading time and obtained the highest PEC of 1.94%. [18]. Wu et al. fabricated graphene nanoflake (GNF) films on a FTO glass using a doctor blade method and achieved 6.08% PCE with thermal annealation of the films in Ar atmosphere [19].

In the present investigation, multiwalled carbon nanotubes (MWCNTs) have been prepared via CVD method using Camphor as carbon source. The influence of temperature on the growth of MWCNTs and their use in ZnO-based DSSC as CE has been reported. The comparison between PEC performance of ZnO-based DSSCs using CNT-based and Pt: FTO based counter electrode is also debated in the present paper.

2. Experimental

2.1 Preparation of MWCNTs by CVD method

Camphor (98%, Aldrich) and ferrocene (98%, Aldrich) of AR grade were used without any further purification. The CVD system comprising of vaporizing and pyrolyzing electrical furnaces was employed for the deposition of carbon nanomaterials, with a quartz tube of length 1m traversing both of them. Nitrogen to

be deposited on the quartz substrates as well thick layer of carbon was also observed on the inner side of the quartz tube. This procedure was repeated by varying the deposition temperature of pyralizing furnace from 700 ^{0}C to 1000 ^{0}C at the interval of 100 ^{0}C. Further the synthesized films were denoted as T_7, T_8, T_9 and T_{10}, respectively.

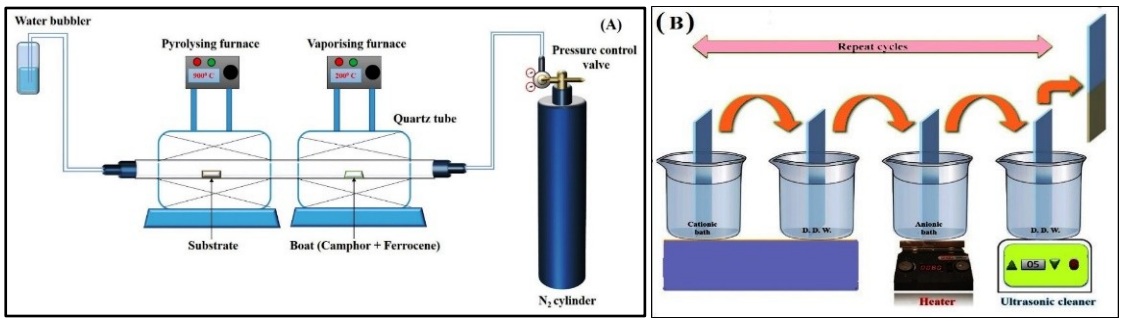

Fig. 1 (A)Schematic of CVD and (B) M-SILAR set up used for the experiment.

2.2 Preparation of ZnO thin film by M-SILAR method

ZnO thin film was deposited using zinc-ammonia complexed precursor as cationic source and 1% diluted hydrogen peroxide kept at 353 K temperature as an anionic source with slight modifications as discussed in our previous report [20]. The schematic setup used for M-SILAR deposition of ZnO thin film is as shown in Fig. 1(B). The M-SILAR cycles were repeated successively for 150 times and the as-deposited film was annealed at 673 K in an air for 1 h to remove the hydroxide phase and to increase the crystallinity.

2.3 Assembly of ZnO-MWCNTs based DSSC

Compact DSSCs were fabricated using a typical two electrode configuration with an active area of 0.25 cm^2. N3 dye-loaded ZnO sample was used as photoelectrode and MWCNTs coated quartz substrate and Pt coated FTO (Pt: FTO) as the CEs. The two electrodes were assembled using a thermoplastic (1mm). The space between the electrodes was filled with an electrolyte comprising of 0.1 M lithium iodide and 0.05 M iodine in acetonitrile through the predrilled hole in the CE.

2.4 Characterization techniques

The structural properties of MWCNTs and ZnO sample was studied by using X-ray diffractometer (XRD) (Philips, PW 3710, Holland) operated at 40 kV, 30 mA with Cu K_α radiation (λ = 1.5406 Å). The surface morphology was observed by means of scanning electron microscopy (SEM) (JEOL JSM -6360, Japan). J-V curves were recorded on the solar simulator (model CT- 150 AAA, photoemission tech, USA) under an Air Mass 1.5 G solar irradiation. Electrochemical impedance study was carried out with electrochemical workstation (AUT85804, Netherlands) using the frequency range of 1MHz to 0.1 Hz in AC mode (100 mV).

3. Results and discussion

3.1 XRD study

Fig. 2 (A) shows the XRD patterns of as deposited MWCNTs with different pyrolysis temperatures from 700 to 1000 ^{0}C. The XRD patterns of all the samples are found to be dominated by strong (002) diffraction peak, which suggests the production of MWCNTs. Also, the hexagonal graphite structure of MWCNTs has been confirmed by matching XRD pattern with JCPDS Card No. 00-008-0415. The interplanar spacing (d) between two graphite layers in the MWCNTs is found to be 3.3500 Å. The result implies that the as-grown film is composed mainly of layered graphite structure [21]. Several characteristic signals of the substrate denoted by asterisks (*) have also appeared in all the spectra.

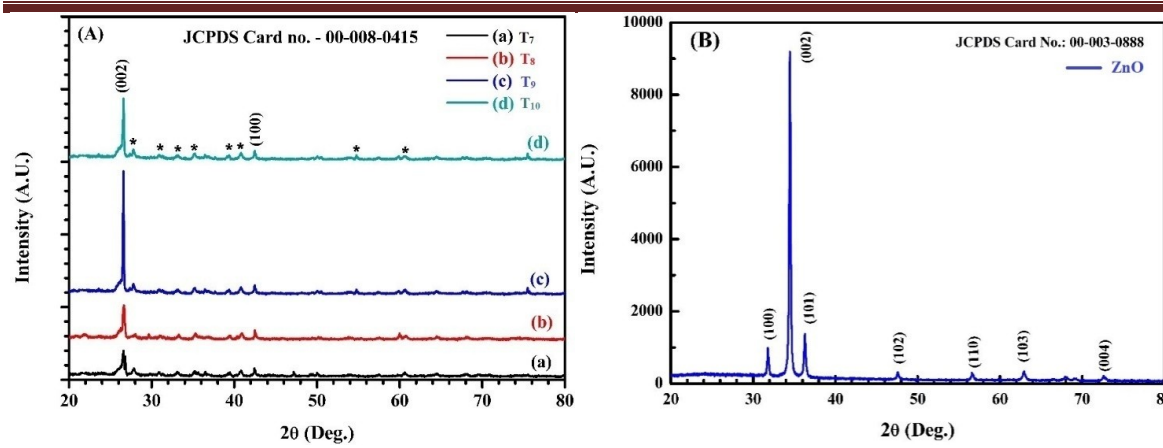

Fig. 2 (A) XRD patterns ofa) T_7, b) T_8, c) T_9 and d) T_{10} samples and (B) XRD spectra of ZnO sample

Fig. 2(B) shows the XRD image of ZnO sample prepared with M-SILAR method. All the diffraction peaks are indexed to a hexagonal wurtzite structure of ZnO, which agrees well with the standard JCPDS data (JCPDS Card No. 00-003-0888). The XRD pattern of the sample is dominated by strong (002) diffraction peak, which suggests that the ZnO nano/microstructure is favorably oriented along c axis [22].

3.2 Scanning Electron Microscopy (SEM)

Fig. 4(a-d) shows the surface SEM images of CVD deposited a) T_7, b) T_8, c) T_9 and d) T_{10} samples grown at different substrate temperatures. The significant effect of variation of substrate temperature on the growth of nanostructure has been clearly observed. The growth of the carbon nuclei on the substrate surface occurs by pyrolytic decomposition of the precursor which gives rise to the formation of a uniform film.

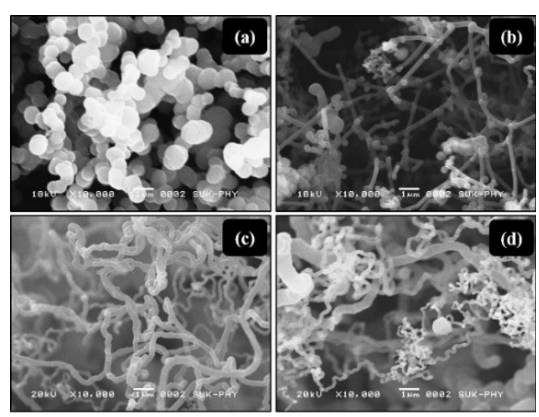

Fig. 4 Surface SEM images of a) T_7, b) T_8, c) T_9 and d) T_{10} samples

The growth temperature has been varied between 700 ^{0}C and 1000 ^{0}C. There is no growth of CNTs at 700 ^{0}C because this temperature is not sufficient to pyrolyze the carbon source. The SEM image reveals the presence of highly dense carbon nanobeads spread all over the substrate surface [23]. At 900 ^{0}C, the quantity of CNT bundles within the sample has decreased (Fig. 4 (c)) and thick nanotubes have been formed. The diameter of nanotubes increased with increasing temperature suggesting an increased mobility of the Fe particles on the quartz tube, leading to larger Fe clusters. At 1000 ^{0}C, a sudden change has been observed in the morphology as well as increase in diameter of the carbon nanotubes is observed.

Fig. 5 (a-d) shows the SEM images of ZnO sample with different magnification (x5k to x50 k, respectively). ZnO microflowers like structure have been observed, oriented perpendicular to the surface of the substrate. Due to the coalescence of the ZnO nanorods, a nuclear reunion may take place and as a result, microflowers like structure have been obtained. The observed microstructure supports well to the XRD study. Microflowers like 2-D surface morphology is beneficial to adsorb more dye molecules due to the large surface area which will increase the power conversion efficiency of the device [24].

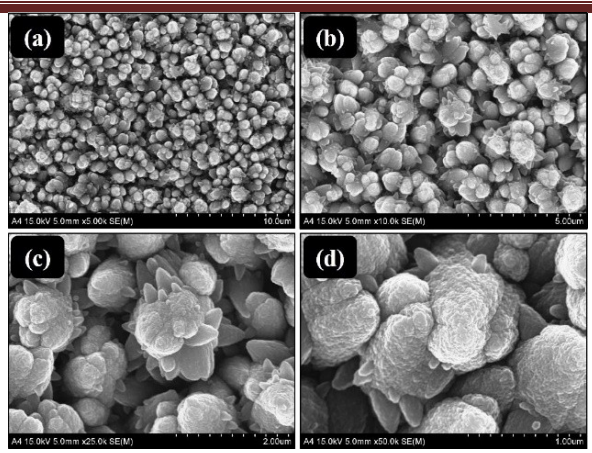

Fig. 5 Surface SEM images of ZnO sample at a) x 5k, b) x 10k, c) x 25k and d) x 50k magnifications

3.3 DSSC performance of CNT and Pt: FTOas counter electrodes

Fig. 7 (A) shows the ideal diode behavior curve due to the establishment of a junction amongst the ZnO photoelectrode, electrolyte and counter electrodes. Fig. 7(B) shows the photocurrent density versus voltage (J-V) curves upon illumination, of the DSSCs prepared using ZnO-Pt: FTO and ZnO-CNT samples, respectively. Table 1 shows the several solar cell parameters for the DSSCs, where J_{sc} is the short circuit current density, V_{oc} is the open-circuit voltage, FF is the fill factor, η is the power conversion efficiency (PCE), R_s is the series resistance and R_{sh} is the shunt resistance. DSSC prepared using ZnO-Pt: FTO sample exhibited the PCE of 0.56%, with an apparently higher J_{sc} of 4.00 mA/cm^2 and V_{oc} of 0.43 V.

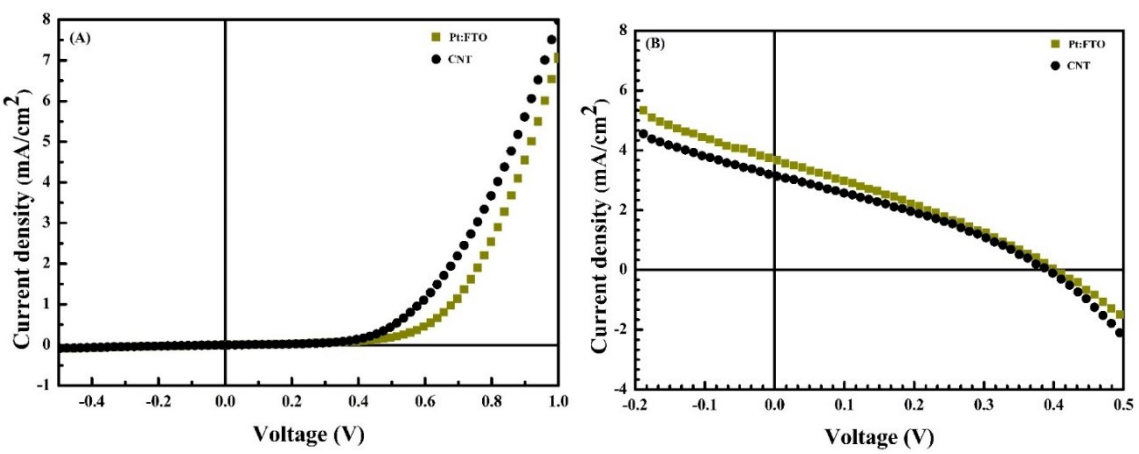

Fig. 7 (A) Photocurrent density- voltage (J-V) curves under dark and (B) upon illumination of DSSCs prepared using ZnO-Pt: FTO and ZnO: CNTsamples

This device contributes better PCE because the Pt possesses a superior catalytic property to CNTs and Pt based counter electrode leads to a lower series resistance R_s and higher shunt resistance R_{Sh} [25-27].

Table 1. Photoelectrochemical performance of the DSSCs prepared using ZnO-Pt: FTO and ZnO: CNTsamples, determined by photocurrent density- voltage (J-V) characteristics

DSSC	Solar cell parameters					
	J_{sc} (mA/cm^2)	V_{oc} (V)	FF	η (%)	R_s (Ω)	R_{sh} (Ω)
ZnO-Pt: FTO	4.00	0.43	0.33	0.56	255	992
ZnO: CNT	3.67	0.40	0.29	0.42	340	436

DSSC based on ZnO: CNT sample showed relatively less PCE of 0.42 % with J_{sc} of 3.67 mA/cm^2 and V_{oc} of 0.40 V compared to that of DSSC based on ZnO-Pt: FTO sample. ZnO-CNT show a comparable photovoltaic performance with the conventional ZnO-Pt: FTO electrode, this could be due to the perfect

combination of superior electro catalytic activity and high electrical conductivity derived from the unique structure of MWCNTs.

4. Conclusions

A simple, economic CVD approach for synthesis of MWCNTs and their use as a CE in the ZnO-based thin film DSSCs has been demonstrated in the present investigation. The formation of hexagonal graphite structure of MWCNTs and hexagonal wurtzite crystal structure of ZnO has been confirmed using XRD analysis. The deposition temperature significantly affects the surface morphology of MWCNTs. 900 ^{0}C is found to be optimum temperature for the growth of MWCNTs using camphor and ferrocene as carbon source and catalyst, respectively. ZnO microflowers like structure has been obtained by M-SILAR method. DSSC prepared using ZnO-Pt: FTO exhibited the highest PCE of 0.56%, with its maximum J_{sc} of 4.00 mA/cm^2 and V_{oc} of 0.43 V than that of ZnO-CNT based DSSC which showed the relatively less PCE of 0.42 % with J_{sc} of 3.67 mA/cm^2 and V_{oc} of 0.40 V. The performance of the ZnO-CNT counter electrode is mostly akin to that of the typical ZnO-Pt: FTO counter electrode. In conclusion, it is seen that MWCNTs can be employed as an effective counter electrode for triiodide reduction in DSSCs. So, CNTs have great potential to use as CE in DSSC.

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STUDIES ON THE CONTACT ANGLE HYSTERESIS OF TRANSPARENT SILICA COATINGS PREPARED BY SOL-GEL PROCESS

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ABSTRACT: *Studies on the contact angle hysteresis of liquids on the silica thin films on glass prepared by different hydrophobic precursors and co-precursors was done. The probe liquid used was water with liquid to vapour surface tension 0.072 N/M. The synthesis of silica thin films on glass was done by the spray technique. The wettability of a solid surface is one of the crucial parameter. For silica thin films, not only the contact angle for self-cleaning applications but also the contact angle hysteresis is important. There is little work done on the study of the contact angle hysteresis of the different polymer surfaces and metallic surface used for the self-cleaning solar panels and windows.*

1.Introduction

In recent years, the study of hysteresis of the contact angle was done worldwide to understand the exact water to solid surface interactions. The study of contact angle is an important factor in the surface science. Contact angle is an essential thermodynamic parameter which often used to quantify the wettability of the solid surface. It is the frequent assess of the hydrophobicity of the solid surface. The literature survey [1-4] it is well recognized that the significant contact angle measurements can be used in the determinations of solid-liquid surface tensions. Many years in the past decades, plenty technique [5] have been used to measure contact angle which was motivated by the thought of using the equation first obtained by Thomas Young in 1805 [6-9]. This Young's equation rules the equilibrium of the three interfacial tensions and the Young contact angle θ_Y of a liquid drop on a solid as per the equation,

$$\gamma_{LV} COS\theta_Y = \gamma_{SV} - \gamma_{SL} \quad (1)$$

Where, γ_{LV} is the liquid-vapour surface tension, γ_{SV} the solid-vapour surface tension, γ_{SL} the solid-liquid surface tension, and θ_Y is the Young contact angle. Young assumes that the solid surface is smooth, homogeneous and rigid; it should also be chemically and physically inert with respect to the liquids to be employed. Typically, according to his equation, a unique contact angle is expected for a given system. In a real system however, a range of contact angles is usually obtained instead. The upper limit of the range is the advancing contact angle, θ_a, which is the contact angle found at advancing edge of a liquid drop. The lower limit is the receding contact angle, θ_r, which is the contact angle found at the receding edge. The difference between the advancing and receding contact angles is known as the contact angle hysteresis, $\theta_{hyst:}$

$$\theta_{Hyst} = \theta_a - \theta_r \quad (2)$$

Basically, all solid surfaces exhibit contact angle hysteresis and because of this hysteresis, the contact angle interpretation in terms of the equation (1) is continuous. All the experimentally calculated or observed contact angles are trustworthy and proper. The contact angle hysteresis has been studied extensively in the past decades. These extensive studies have directed contact angle hysteresis to surface roughness [10-15] and surface heterogeneity, as well as metastable energy states [16-18]. Some observed that hysteresis decreases with increase in molecular volume of the liquid on monolayers [19-20]. Also from the literature, contact angle hysteresis was found to be related to molecular mobility and packing of the surface, liquid penetration and surface swelling [21-22]. Studies have been shown that the contact angle hysteresis strongly depends on the liquid molecular size and solid/liquid contact time [23-24]. These extensive conclusions lead to the belief that liquid sorption and liquid retention are causes of contact angle hysteresis. Hence, the current research work is based on the observations of the determining the true contact angle and contact angle hysteresis of water droplets on the silica glass thin films. These silica films were prepared by two stage sol-gel process and dip coating by using different hydrophobic precursors in order to interpret the correct contact angles on silica films.

2. MATERIALS AND METHODS:

The synthesis of silica thin films was done by using the chemicals methyltriethoxysilane (MTES), methyltrimethoxysilane (MTMS), phenyltrimethoxysilane (Ph-TMS), Iso-butyltrimethoxysilane (ISO-BTMS) (Sigma-Aldrich Chemie, Germany), as precursors and methanol (S.D. fine Chem. Ltd, Mumbai, India) as a solvent, ammonium fluoride (NH_4F) (Thomas Baker, Mumbai, India) as catalyst. The glass substrates were cleaned by the procedure described earlier [25].

3. RESULTS AND DISCUSSIONS:

1] FOURIER TRANSFORM INFRARED SPECTROSCOPY:

From the FTIR spectra several characteristic deeps were confirmed in the range of 500 to 4000 cm^{-1} signifying the presence of non-polar methyl groups in the silica thin films. From the figure 1, the FTIR spectra of the silica thin films synthesized from the different hydrophobic precursors are shown. Since, all the hydrophobic precursors are containing non-polar methyl groups and hence the particle sizes of the silica thin film are larger enough to reflect the EM radiation in the visible region. From figure a, the spectrum signifies the functional groups of the methyltriethoxysilane (MTES) precursor. In the MTES based silica thin films the non-hydrolysable ($-CH_3$) groups are responsible for the hydrophobic property of the thin films. Figure a, depicts FTIR spectrum in which the presence of the C–H, Si–C bonds at 2900 cm^{-1}, 1250 cm^{-1} respectively and which reveal the extent of hydrophobicity. Figure b, depicts FTIR spectrum of the MTMS precursor which has strong deeps of the C–H, Si–C since it contains more non-polar methyl groups than MTES as it is lucid from the FTIR spectra. Figure c, depicts the spectrum of phenyltrimethoxysilane (Ph–TMS) based thin films clears the C–H, groups in the thin films. The absorption deep observed at 1500 cm^{-1} is corresponding to the Si-Phenyl groups of the thin film. Figure d, depicts the spectrum of isobutyltrimethoxysilane (Iso-BTMS) based thin films. The FTIR spectra contains the deeps observed at the around 1000, 800, and 500 cm^{-1} are due to the asymmetric, symmetric and bending modes of the silicon dioxide respectively [26].

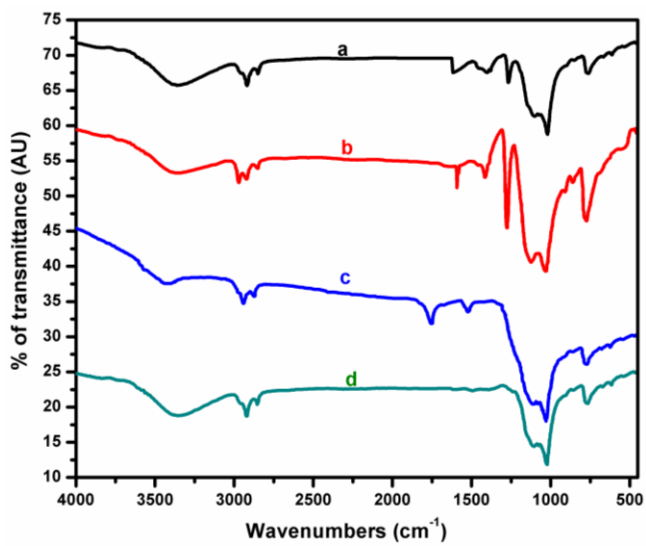

FIGURE 1: FTIR SPECTRA OF THE SILICA THIN FILMS

2] CONTACT ANGLE HYSTERESIS:

The wettability of the silica thin films was quantified with the water droplet of 10 µL volume using the Rame-Hart goniometer. Surface hydrophobicity is usually determined by measuring the contact angle (CA) of water droplet on a surface to be tested. The angle between the surface and the water meniscus near the line of contact, measured from inside the liquid droplet, gives sign of the wettability of the surface. In this method the volume of the water droplet can affect the contact angle. There is usually a difference between the angles produced as the volume of the drop is increased as (the advancing angle) and that when it is decreased (the receding angle). This difference, termed as the contact-angle hysteresis, gives a measure of the surface 'stickiness'. The greater this difference (larger hysteresis) the more water drops will stick to the surface. Usually low hysteresis is desired when dealing with superhydrophobicity of the surfaces. This means water droplets will roll off extremely easily. Theoretical equilibrium angles lie between the

advancing and receding angles, sometimes being determined by vibrating the drop. The water drop contact angles of the silica thin films are as shown in the figure 2 (A-D). From figure 2 A, is the CA image of the MTES based silica thin film, 2B, is the CA image of the MTMS based silica thin film, 2C is the CA image of the Ph-TMS based silica thin film, 2D is the Iso-BTMS based thin film as shown in the figure.

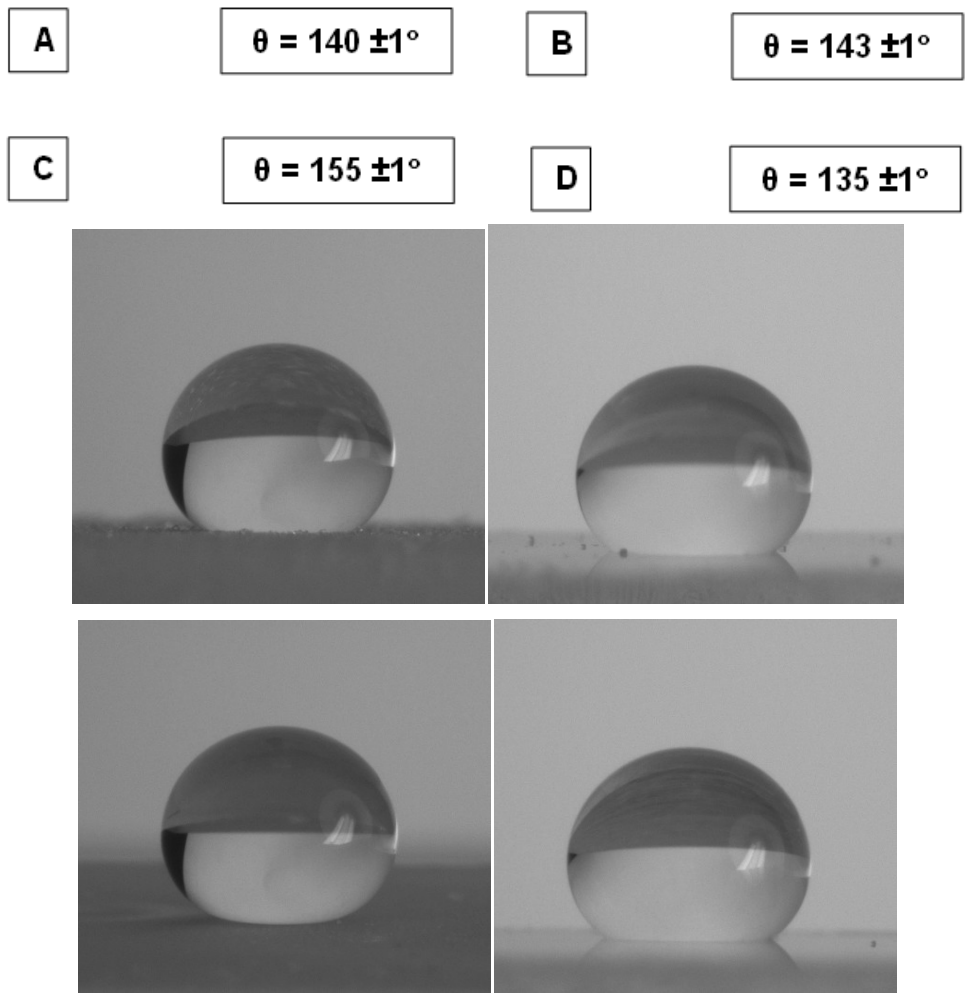

FIGURE 2 (A-D): CA IMAGES ON THE SILICA THIN FILMS

Table no: 1 depicts the contact angle hysteresis of the synthesised silica thin films.
From the table it is clear that the CA hysteresis for the silica films is very low and which is well analogues with the literature survey.

TABLE 1: CONTACT ANGLE HYSTERESIS OF WATER DROPLET ON THE SILICA THIN FILMS

Sr. No.	silica thin films	Static Contact angle (θ= ±1°)	Advancing Angle (θ= ±1°)	Receding angle (θ= ±1°)	CA Hysteresis (θ= ±1°)
1	MTES	140	142	138	4
2	MTMS	143	145	141	4
3	Ph-TMS	155	156	154	2
4	Iso-BTMS	135	136	133	3

CONCLUSIONS:

The contact angle hysteresis i.e. the deviation from the true contact angle between the solid and the liquid plays a significant role in quantifying the behaviour of non-wettability of the hydrophobic surfaces. The current research work has been demonstrated that the non-wettability characteristic in the silica thin

films can be easily obtained from the hydrophobic precursors by using sol-gel route. If the solid surface energy is high it will attract the water molecules via van der Waals forces of attractions and it is called as the hydrophilic surfaces which gets wet easily. Generally, for the superhydrophobic surfaces the contact angle hysteresis was found to be very low and the same demonstrated here.

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ELECTROMAGNETIC ABSORPTION PROPERTIES OF POLYPYRROLE/POLYANILINE COMPOSITE THIN FILMS

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ABSTRACT: *In present paper Polypyrrole (PPy)/Polyaniline (PANI) composite thin films were prepared by simple chemical bath deposition method on stainless steel substrate. Deposited composite thin films were further characterized for various characterizations like XRD, SEM, FT-IR, and microwave absorbing and electromagnetic shielding properties. The XRD analysis reveals that, polypyrrole/polyaniline composite thin films amorphous nature. Surface morphology of composite thin film shows the fiber like morphology. The strong absorption bands in the FT-IR spectra confirm the formation of PPy/PANI composite. The microwave absorption properties like shielding effectiveness and reflection loss has been measured in the frequency range 12-18 GHz in Ku band; the maximum shielding effectiveness -19.58 dB is observed for 17.7GHz.*

Keywords: *Chemical bath deposition, XRD, FT-IR, SEM, Microwave properties*

I. Introduction

Now a day's microwave-absorbing material have attracted much attention in research from last few decades, due to their significant role in promising application in military stealth technology and electromagnetic shielding technology for personal protection. The important key requirements for good microwave absorbing materials include the wide absorption bandwidth, strong attenuation property, lightweight, and low thickness or thin. However, these properties are unlikely to occur in single material so there is need to study the composite materials.

Polymers have attracted attention for many applications in EMI shielding [4], battery[5], supercapacitor [6], chemical sensor [7], biosensor [8], nanocomposite [9], light-emitting-diode [10], electrochromic display [11], Out of several conducting polymers the Polypyrrole and Polyaniline are the most promising materials due there following properties like, easy of synthesis, good environmental stability, good mechanical and electrical properties, high conductivity, loss dielectric loss. Etc [2]

There are several deposition methods have been employed for the synthesis, here we have used a simple and cost effective chemical bath deposition method. The stainless steel was used as a substrate for the deposition. Since stainless steel is cost effective and useful material in the radio frequency and microwave region [9]. In this paper we have reported the microwave properties of PPy/PANI composite thin films deposited by chemical bath deposition method on stainless steel substrate in the frequency range 12-18GHz.

II. EXPERIMENTAL

All AR grade chemical were used for the synthesis Aniline Pyrrole, Ammonium peroxydisulphate (APS), and sulphuric acid (H_2SO_4) were used as a received. Pyrrole monomer (0.17ml) and aniline monomer (0.22ml) dissolved in 25 ml distilled water separately in the presence of sulphuric acid and APS (1.329gm) in 25ml distilled water.

The PPy/PANI composite thin film was prepared by a chemical bath deposition technique on stainless steel substrate. The dimensions SS substrate of thickness ~0.5mm was cut to the size of 1.1×5 cm. In CBD, the substrate were polished to smooth surface finish using finger grades of polish paper, washed with soap solution and distilled water and dried under a hot air stream and wiped by tissue paper. Here oxidant to monomer ratio (50-50)%; the SS substrate were immersed in the bath at room temperature with stirring. During the precipitation occurred and the deposition of PPy/PANI took place on the substrate. The substrate coated with PPy/PANI thin film during the time interval at 30-60 min for SS on adherent bluish black colored. While the increasing the ratio of PPY the film become black. Then films washed with distilled water, dried in air.

III. RESULTS AND DISCUSSION

3.1 XRD (X-Ray Diffraction) Study

The XRD pattern of the prepared film was obtained with Bruker D2Phaser X-Ray diffractometer using $Cu-K\alpha$ radiation (λ=1.5418 °A). Scanning was carried out in the 2θ range from 0° to 90° at a scan rate of 10° per minute. Fig. shows the XRD pattern of PPy/PANI thin film. XRD analysis revealed that the PPy/PANI composite film shows amorphous nature.

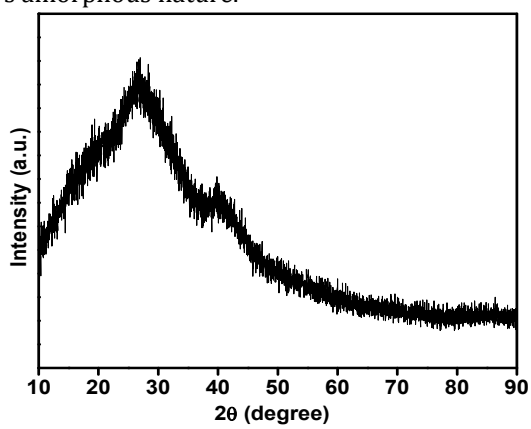

Fig. 3.1. XRD pattern of PPy/PANI composite sample deposited with CBD for 1hour reaction time

3.2 Scanning Electron Microscopy (SEM) study

The JSM-6360 JEOL, Japan scanning electron microscope was used to study surface morphology of the samples. Surface morphology of polypyrrole, polyaniline and composite of polypyrrole/polyaniline is as shown in fig.3.2from the figure it is seen that polypyrrole is a fiber like structure having presence of spherical nanoparticles at the surface while polyaniline thin film also shows similar type of morphology whereas PPy/PANI composite thin film clearly shows compact morphology also having fiber like structure covered with spherical nanoparticles.

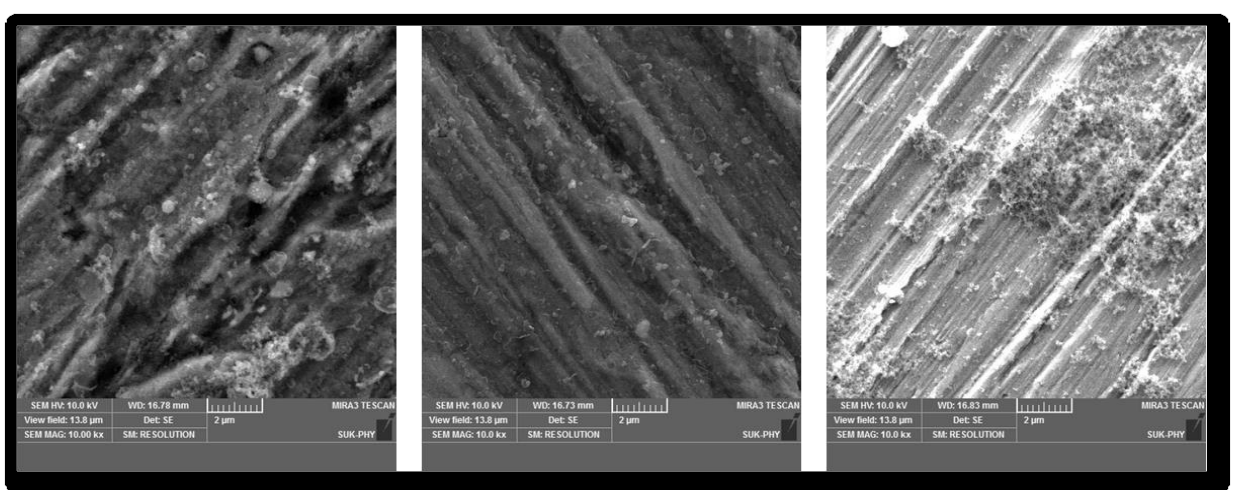

Fig.3.2 Surface SEM images of a)PPy b)PANI and c) PPy/PANI composite in CBD method for 1h time.

3.3 FT-IR Spectroscopy

The FTIR spectrum of polypyrrole /polyaniline (PPy/PANI) composite thin film was recorded between 4000cm^{-1} to 500cm^{-1} at a spectral resolution of 2cm^{-1} on a Perkin Elmer 1710 spectrophotometer. There are many pecks are observed in this composite thus plane deformation found at 1034 cm^{-1}. The N-H bending occurred at 1293cm^{-1}. The most intensive pecks are observed at 1482 cm^{-1} and 2353 cm^{-1} are attributed for C =C stretching vibrations while band observed at 2926 cm^{-1} depicts C-H stretching. The presence of these pecks confirm the formation of polypyrrole/polyaniline (PPy/PANI) composite structure.

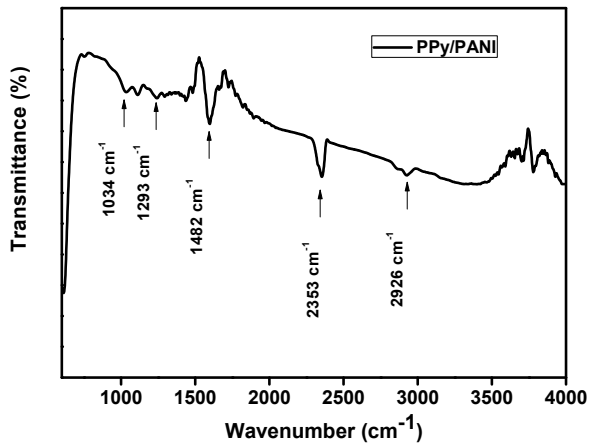

Fig. 3.3 FT-IR of PPy/PANI composite sample deposited with CBD method for 1hr time

3.4 Microwave Properties

3.4.1. Reflection loss

Reflection loss (RL) study has been carried out in the frequency range 12-18 GHz to compare and evaluate the microwave absorption properties of PPy/PANI thin films deposited on alumina substrate. For the measurement of RL value samples were assembled in the Ku-band waveguide connectors and joined to the vector network analyzer by using coaxial cable. Fig 3.4.1 illustrates the variation of reflection loss (with dB as the measuring unit) with frequency. RL value of PPy/PANI was calculated from the complex scattering parameter (S^*_{11}), which was obtained directly from Agilent Vector Network Analyzer PNA 5230 and it can be expressed as,

$$RL = 20 \log |S_{11}| \quad \text{..... ....(1)}$$

In the present study, it was found that PPy/PANI thin films shows maximum RL value up to -19.58 dB i.e.(>95.0% microwave power absorption) at 17.7 GHz. Also at frequency 12.5 and 15.1 GHz the absorption peaks crosses the -10dB level. So it is clear that PPy/PANI composite thin film shows good microwave absorption properties in Ku band region. It is well accepted that the thickness of absorbent plays a vital role to enhance the microwave absorption phenomena and frequency position of microwave absorption. RL value is relatively high with a matching thickness of film is 6.1 μm

When measurements are made with a network analyzer, the total shielding effectiveness can be conveniently express in terms of the S-parameters as,

$$\mathbf{SE}_T = \mathbf{20 \log} \ |S_{21}| \quad \text{..... ....(2)}$$

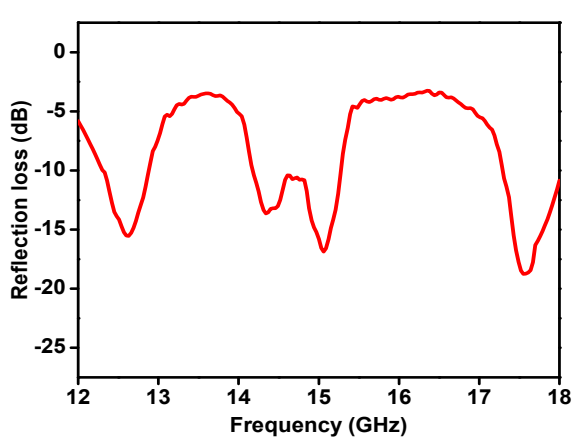

Fig.3.4.1.Reflection loss of PPy/PANI composite sample deposited with CBD method for 1hr time

Shielding is conductive obstruction enveloping an electrical circuit or device to provide isolation. The SE is a measure of the material's ability to attenuate the intensity of EM waves. The sample having greater SE value in decibels lowers the energy passing through that sample. EMI shielding mechanism

primarily depends on the absorption of the EM energy, reflection from the material's surface, and multiple internal reflections of the EM radiations. For having good shielding behavior it is necessary that material should possess electrical conductivity in the range 10^{-4} to 10^{1} S/m.

3.4.2 Shielding effectiveness

Fig.3.4.2 depicts the variation of total shielding effectiveness (dB) with frequency in 12-18 GHz range for PPy/PANI composite thin film. The
electrical conductivities of the PPy/PANI thin films is 5.8×10^{-4} S/m. It is seen from figure that PPy/PANI thin film shows good shielding properties. The maximum SE value observed is -16 dB at 16.2 GHz while at 13.8 GHz the observed SE value is -14 dB.

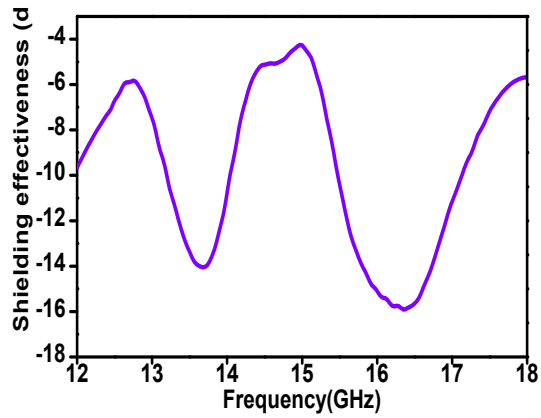

Fig.3.4.2Shielding effectiveness of PPy/PANI composite sample deposited with CBD method for 1hr time

4. CONCLUSIONS

In this paper, the PPy/PANI composite thin film were synthesized by chemical bath deposition technique on stainless steel substrate. The composite of polymers is observed that amorphous nature and influence on the morphology. The IR spectrum shows the absorption peaks of composite formation. The PPy/PANI composite thin film shows that maximum reflection loss up to -19.58dB at 17.7GHz and absorption pecks crosses the -10dB level at 12.5 and 15.1GHz frequency range and good shielding properties of PPy/PANI composite thin film. Stainless steel is promising material for variety of applications in microwave antenna applications the reflection, absorption is very important.

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Development of $Cu_xFe_{2-x}O_3$ as a gas sensor by facile combustion route

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ABSTRACT: *Facile synthesis of Cu substituted Fe_2O_3 was employed using Auto-combustion method. X-ray diffraction (XRD) was employed to characterize the structure of the material. The effect of Cu doping on the crystalline structure and gas sensing properties of the final product have been investigated. The gas sensing properties of $Cu_xFe_{2-x}O_3$ (x=0,0.1,0.2,0.3) materials for semiconductor gas sensors are presented. For composition with x=0.2(S_3), sensor exhibited highest response (62%) towards acetone vapor at an operating temperature 350°C. The response and recovery time of the sensor for x=0.2 were quite low (15sec and 48sec) respectively.*

Keywords: *Facile Synthesis, Selective acetone sensor.*

I. Introduction

A Iron oxides and oxide-hydroxides are widespread in nature, play an important role in many geological and biological processes, and are widely utilized by humans, e.g., as iron ores, pigments, catalysts, in termites. Due to the interesting physical properties of Fe_2O_3, it acts as a potential applicant for future technological applications [1,2]. For the formation of Fe_2O_3nanoparticles we adopted the superficialCitric acidcombustion method[3,4]. In this study, we discuss the effect of Cu substitution on the structural and gas sensing properties of Fe_2O_3.

II. Experimental details

The synthesis of the Fe_2O_3 was prepared by an auto combustion method using metal nitrates of starting materials and Citric acid as a fuel. The Ferric nitrate $Fe(NO_3)_3.9H_2O$,Cupric nitrate $Cu(NO_3)_3$. $3H_2O$ and Citric acid ($C_6H_8O_7 \bullet H_2O$) were dissolved in 100 ml distilled water with magnetic stirring for 30min at 70°C constant temperature. These metal nitrates and fuel were used without any further purification system. Then, the mixed solution was poured into a dish and heated at 100 °C to transform into a dried gel.

Being ignited, at the end combustion takes place and reddish powder is formed. This powder was quenched to room temperature and ground thoroughly. This process has been repeated several times in order to achieve homogenous ultrafine powder. The final powder was calcined at 600°C for 2 hrs and pelletized using freshly prepared poly vinyl alcohol (PVA) as a binder. Cylindrical pellets having average dimension 13mm diameter and 2 mm thickness were prepared using hydraulic press. These pellets were sintered at 600 °C for 4 hrs by slow step sintering schedule to carry out its gas sensing properties. In order to form copper ferrite powders with compositions of ($Cu_xFe_{2-x}O_3$ (where x=0.1,0.2,0.3) were synthesized via above-mentioned process and then allowed to cool down to room temperature naturally.The resistance of the sample was measured by two-probe dc measurement technique and the sensitivity of sensor for reducing gases was calculated by following formula as:

$$S(\%) = \left[\frac{Rg - Ra}{Ra}\right] \times 100$$

Where, R_a is resistance of sensor in air and R_g resistance after the exposure of test gas.

III. Results and discussion

The XRD patterns of the products synthesized with different amounts of Copper are shown in fig. All the diffraction peaks are sharp and can be perfectly indexed to hexagonal phase Fe_2O_3 (JCPDS card no.33-0664). All the diffraction peaks can be indexed as hexagonal Fe_2O_3 with lattice constants a= b=5.03438 Å, c= 13.7559 Å.

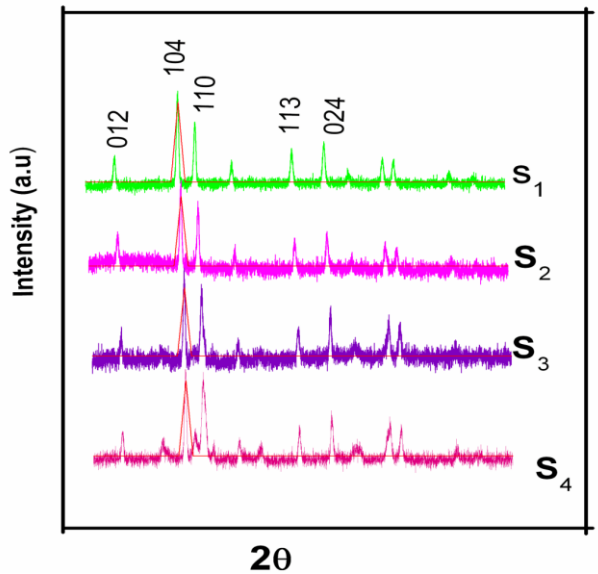

Figure 1. XRD pattern of $Cu_xFe_{2-x}O_3$sintered at 600°C

In the present work, we developed a gas sensing unit for gas sensitivity of the Fe_2O_3 sensors measurement.It consists of a horizontal coaxial tube is inert in furnace .The temperature of gas sensing unit controlled 375°C to take measurement of sample. We use two probe methods, conducting silver paste use for ohmic contact resistance. This sample is inserting in horizontal coaxial tube to take gas sensing response reading of sample at temperature range 350°C to 200°C every 25°C temperature interval. The known amount of gas injected in gas sensing system measure the gas sensing reading. Sensor responses of the sample pure, 0.1Cu,0.2Cu,0.3Cu to gases at various temperatures as shown in fig. gas sensitivity at optimum temperature 350°C. As can be observed from samples behave certain responses at all different testing temperature. The responses of all the sensors become increase & increase with temperature increasing & exhibit the max. at 350°C, but show a downward trend at latter max. temperature. As per the observation the optimized gas is acetone which shows the maximum response for the sample at temperature 350°C .The highest responses of 0.2 Cu are 62 %, respectively. Fig. shows that the samples exhibit critically larger response to acetone indicating that sensor performs a good selectivity to acetone gas. However the sensing response for 0.2 Cu to acetone is higher as compared to pure, 0.1 Cu,0.3 Cu.

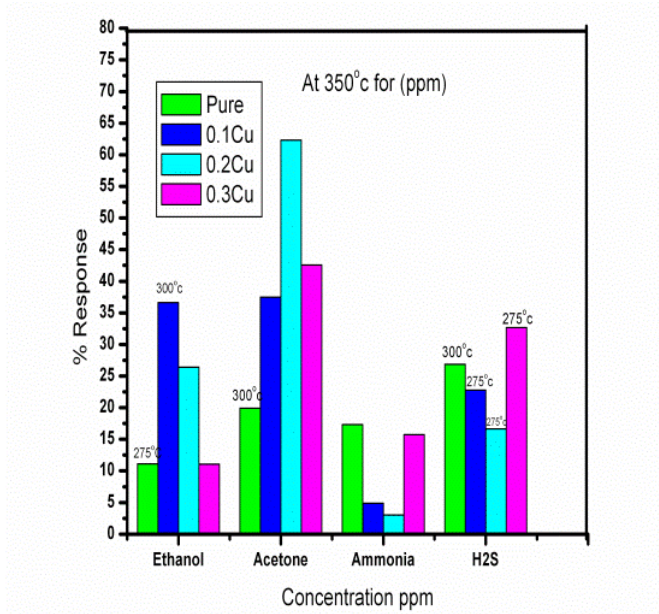

Figure 2. Selectivity of the $Cu_xFe_{2-x}O_3$ sintered at 600°C

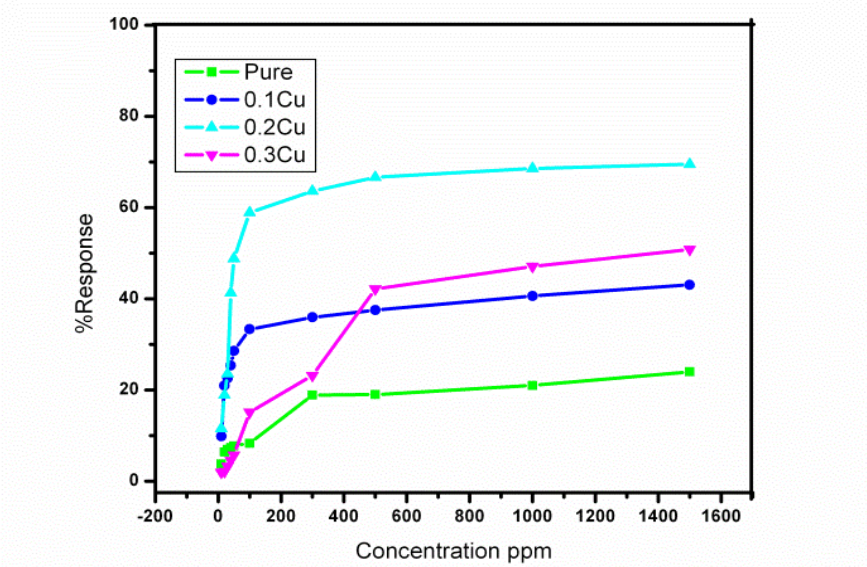

Figure 3.Concentration variatedgas sensing properties of $Cu_xFe_{2-x}O_3$

Sensor response to acetone at optimize temperature for various concentrations from the curve it can be obviously known that resistance of the sensor is reproducible. For repeated testing cycles. Fig shows sensitivity of sensor under different gas concentration at operating temperature 350°C.The sensitivity increases linearly with no sign of saturation as the concentration increases from 10-1500 ppm.

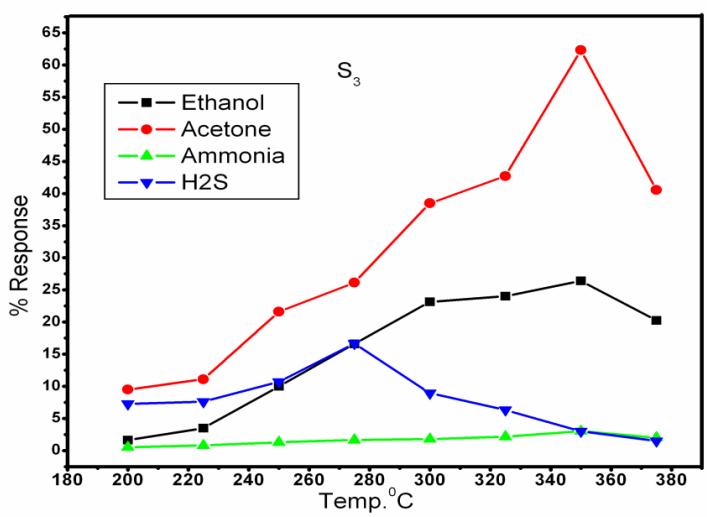

Figure 4. Gas sensing properties of $Cu_xFe_{2-x}O_3$

The S3sample shows selective highest response (62%) for acetone gas at optimal operating temperature 350°Cas shown in figure. The response and recovery time observed in S_3sample were quite low (15sec and 48sec) respectively.

Conclusion

In summary, we have developed a simple one-step facile combustion route for the synthesis of pure and Cu-doped α-Fe_2O_3. The results of X-ray diffraction indicated that Cu ions entered into the crystal lattice of α-Fe_2O_3. As such pure and doped were used as the sensing materials of gas sensors. An enhanced sensing property to acetone was demonstrated, in comparison to the pure sample. In gas sensing measurements 350°c is the appropriate temp to for Cudoped Fe_2O_3 shows maximum response about 62% at 350°C.

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A STUDY OF SILICON DIOXIDE NANOWIRE BY MOLECULAR DYNAMICS SIMULATIONS: INFLUENCE OF INTERATOMIC POTENTIALS AND BOUNDARY CONDITIONS

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ABSTRACT: *The results of molecular dynamics (MD) simulation depend on the choice of interatomic potentials, force conditions and simulation parameters. Interatomic potential has been used for MD simulations of silicon dioxide, based on properties of heat conduction phenomena. In this method, BKS potential is used. Nonequilibrium molecular dynamics method is used to calculate temperature gradient.*

Keywords: *Thermal conductivity, Molecular Dynamics simulation, nanowire*

I. Introduction

In the development of nanotechnology, thermal properties of nonmaterial's is essential part. System failure will happen because of excessive temperatures and temperature gradient. At nanometric scales, for thermal conductivity measurements modeling and simulation tools are necessary. In such a system prediction of heat transfer is challenge when the mean free path of heat carriers is of the same order of magnitude as the thickness of the layers in the material. Molecular dynamics (MD) is a convenient tool for studying atomic scale material. For transport properties, MD is well-suited. (1)

In solids, heat conduction can occur through lattice vibrations, electronic excitations and radiative processes. In insulators, electronic contributions to thermal conductivity are small, At very high temperatures, radiative contributions become important because of T3 dependence of the radiative term. (Young-Gui Yoon, 2004)

As the size of the electronic circuits decreases, density increases and correspondingly dissipation of heat increases. For the dissipation of heat generated in electronic devices metallic heat sink materials such as copper are used. The thermal conductance at interfaces is low due to effective contact area, however this low effective contact area is due to the fact that solid surfaces are rough at atomic level. To increase the effective contact area and hence the interfacial thermal conductance, in the electronic industry, thermal grease is used which is an extremely flexible material that can form an efficient contact with both solid surfaces under pressure, effectively increasing the contact area between the original two solid surfaces. (3)

Due to nanotechnological advances, there is possibility of synthesizing and designing materials at the atomic level. For optimization of design before synthesis, Prediction of the properties of potent materials is allowed by Molecular dynamics (MD) simulations. In many applications, low thermal conductivity materials are required. By introducing porosity thermal conductivity of a solid can be reduced but it reduces the strength of the material. Amorphous materials have low thermal conductivities, but in some applications it is necessary to use a crystalline solid. (4)

Method: The thermal conductivity κ is the ratio of the thermal current(heat flux per unit area) to the temperature gradient along the z direction (Fourier's law)

$$\kappa = \frac{J_z}{\partial T / \partial z} \qquad \text{Eq. a)}$$

Thermal conductivity is calculated by equilibrium molecular dynamics, Nonequilibrium molecular dynamics In equilibrium molecular dynamics, Heat flux autocorrelation function is used while in Nonequilibrium molecular dynamics, temperature gradient is used. In equilibrium molecular Green-Kubo formalism is used while in Nonequilibrium molecular dynamics, Fourier's law is used.

According to the Kinetic theory, the thermal conductivity is given by the formula (due to Finite size effects)

$$\kappa = \frac{1}{3} C v \ell \qquad \text{Eq. b)}$$

Where C is heat capacity per unit volume, v is velocity and ℓ is mean free path of the phonons.

Because of localization effect,care should be taken ,while applying the formula to the glasses. As the energy E of the mode increases, the scattering rate increases as E^4 until $\ell^{-1} \sim k$ where k is the wavevector of the mode. (5)

Heat capacity C is due to contribution of transport phonons, υ and ℓ are the characteristics of "propagative" phonons which are also contributing to the transport properties. (Jullien)

II. Procedure:

A molecular dynamic simulation is performed on a silicon dioxide nanowires using BKS interatomic potential. In simulation box, heat transport coefficients are directly calculated. Heat flux is induced in the system with the help of "hot" and "cold" plate. This creates a temperature gradient and to determine thermal conductivity steady state have to be reached. Plates are compatible with periodic boundary conditions.

In this simulation, a long simulation cell is divided into a series of parallel slabs and the temperature is measured. In the center of the simulation cell, Heat is added to the slab and removed from a slab at one end of the simulation cell at fixed rates by rescaling velocities at every time step. After sufficient time, a temperature gradient is established that decreases from center to ends of the simulation cell. Since a heat flux is given, the temperature gradient is measured, we calculate the thermal conductivity through Eq.1.

The classical BKS interatomic potential is given as

$$\Phi_{ij} = q_i q_j / r_{ij} + A_{ij} \exp(-b_{ij} r_{ij}) - C_{ij} / r_{ij}^6 \qquad \text{Eq.1}$$

Where r_{ij} is the separation between atoms i and j

Φ_{ij} is the interaction energy of atoms i and j, which consists of a Coulomb term and a covalent contribution. (7)

The scope of the paper is to find out the capability of NEMD simulation to provide estimation of the thermal conductivity in SiO_2. Thermal transport is calculated along (001) axis of quartz. Periodic boundary condition is applied along x axis only (for nanowire)

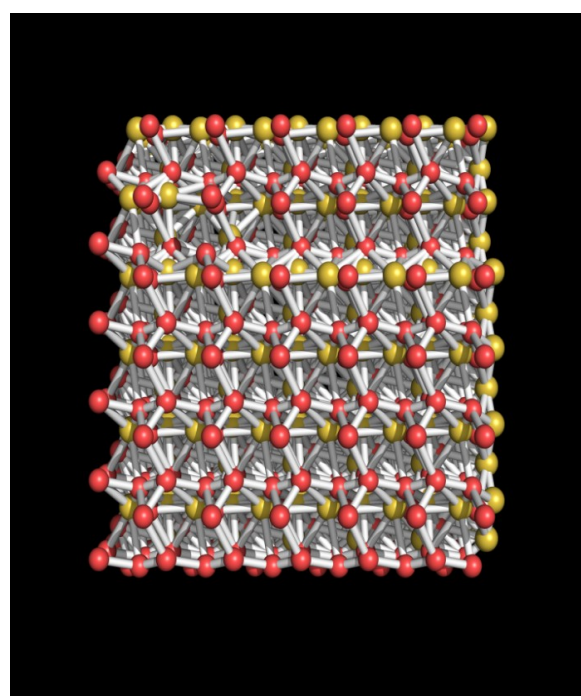

Figure 3: Initial Position of Silicon Dioxide molecule.

III. The model Set up:

This simulation is carried out using classical Molecular dynamic Simulation in a micro canonical ensemble using 1200 particles of Quartz with the help of BKS potential. These particles are packed in a cubic box of length 25.774548A^0 Periodic boundary conditions are imposed along (001) direction. Due to the periodic boundary condition imposed on system, the heat flows from the hot energy reservoir to cold energy reservoir at the end of the silicon slab.MD steps at a constant temperature of 300 K using Anderson thermostat with a time step of t=1.0 picosecond.

Most commonly used theoretical method for molecular dynamics simulation is Non-equilibrium

molecular dynamics (NEMD) to determine thermal conductivity. Ratio of heat flux to temperature gradient gives thermal conduction, There are two approaches to calculate this.

- A known heat flux is imposed and temperature gradient is calculated.
- A known temperature gradient is imposed and heat flux is calculated.

For the imposition of heat flux, simulation cell is divided into even number of equal of section, one half as hot section and other half as cold section

Heat leaves both sides of the hot sections and enters through both sides of cold sections which leads to generate heat flux and corresponding temperature gradient is measured. (8)

Two temperature gradients of equal in magnitude but having opposite sign which is due to periodic nature of the simulation cell. The difference in average temperature of symmetrically equivalent sections

3.1 Pair radial distribution functions (rdf's): For this, parameter rcut is taken as 5.0 and no of time steps from which output is recognized is taken as 100. In silicon dioxide nanowire Si-O distance is linked at corners and in rings 6 members forming the ribs of fused cage-like structure. From fig ,it is seen that the first Si-O peak is broader in this models. The BKS model shows higher coordination numbers at next nearest neighbor distances which is consistent with the higher density obtained wih this potential

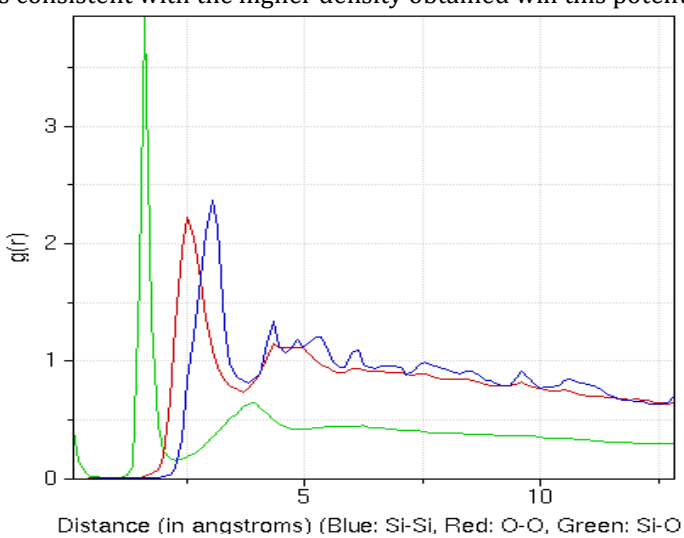

Figure 4: Radial distribution function versus no. of time steps of MD

3.2Temperature versus no of time steps of MD: For the calculation of temperature profile, Temperature gradient is calculated. Heat is added to the slab from the center of simulation cell and removed from slab from other end by Anderson thermostat. This simulation is carried by using microcanonical ensemble (NVE) which shows constant number of atoms, constant volume and constant energy. Total number of time steps is 1500 and equilibrium is attained after750 time steps. initially it shows more fluctuations in temperature but after some 200 time steps it goes down and then attain equilibriums.

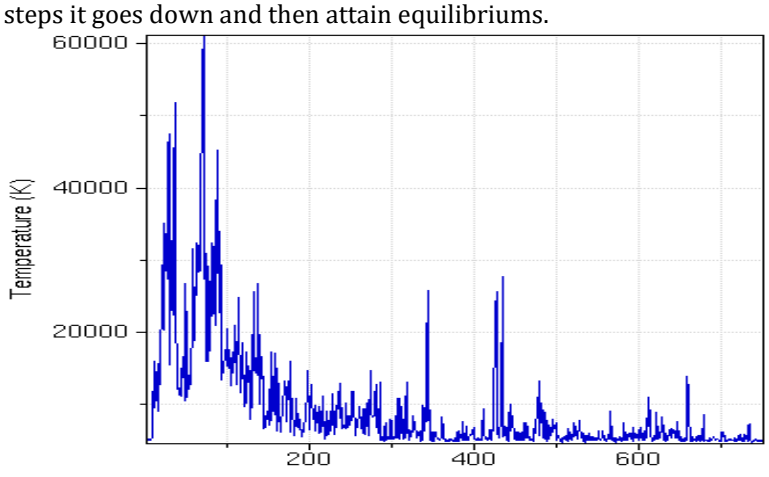

Figure 3: Graph of temperature versus No of timesteps of MD

IV. Results and Discussion: Figure 1 shows initial position of silicon dioxide structure in which 1200 atoms are there in which 400 are of silicon(coloured yellow) and 800 are of oxygen(coloured light red).this structure has 25.774548e-10 x 25.774548e-10 x 25.774548e-10 lengths along x,y and z axis respectively. In this simulation interatomic potential used is BKS which is very effective at low pressures. Figure 2 gives radial distribution function because number of bins used to study are 100 and there are 6 cell-list linked cells. Radial distribution of Si-O bond is wider (green color) in the sense that it gives higher density. Figure 3 shows temperature profile of silicon dioxide with respect to number of time steps. Because of temperature gradient initially it shows maximum temperature but as equilibrium is attained it goes down.

V.Conclusion: A study of molecular dynamics simulation is done with the help of software MD simulation. (9) In this simulation, parameters which are necessary to calculate thermal conductivity are studied here temperature gradient is imposed. Noneqilibrium molecular dynamics simulation method (NEMD) method is most powerful, At the level of nanostructure, properties of materials are changed because of reduction in surface to volume ratio but the properties of silicon remained as it is so as to further use of silicon in nanoelectronics, many nanostructures are to be under study.

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Permeability of nanoparticle sized copper cobalt ferrites

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ABSTRACT: *The polycrystalline aluminium substituted nano-particle sized copper cobalt ferrite samples $Cu_xCo_{1-x}Fe_{2-2y}Al_{2y}O_4$ (where x= 0.0, 0.2, 0.4, 0.6, 0.8, 1.0; y = 0.05, 0.15 and 0.25) have been prepared by standard ceramic technique. Phase formation is investigated using X-ray diffraction, Infrared absorption technique and Scanning electron microscope technique. The lattice constants of the all samples are evaluated from x-ray diffraction data. A universal testing machine as well as Archimedes's method was applied for determining the physical properties of the samples. Ionic radii R_A and bond lengths (A-O) on both sites are found decreases with Al^{3+} and copper content. The Lattice constant 'a', physical density as well as X-ray density of samples goes on increasing with Al^{3+} and copper content. The ratio c/a is found increasing when addition of copper content and decreases with aluminum content. It means that Al^{3+} and copper acquire the tetragonal prolate type distortions on B site and hence (c/a) ratio increases and automatically crystal lattice turned from tetragonal spinel to cubic spinel. The initial permeability decreases with increase in frequency.*

Keywords: *polycrystalline, standard ceramic technique, susceptibility, Curie temperature.*

I. Introduction

In a way, every material utilized today is a composite. Composite materials are a physical mixture of two or more compatible micro or macro constituent particles which differ in form and chemical composition and are essentially insoluble in each other. Composite materials are best suited for scientific applications which could not be achieved by any one component acting on its own. Ferrite / ferroelectric composites are termed as magneto electric (ME) composites due to the coupling between the electric and magnetic fields in the materials. The conversion of magnetic to electric fields in such ME composite originates from the elastic interaction between ferrite and ferroelectric subsystems [1]. In the presence of the magnetic field, the magnetostriction in the ferrite phase gives rise to mechanical stresses that are transferred to the ferroelectric phase, resulting in electric polarization of the ferroelectric phase owing to its magneto electric effect. ME materials find applications as smart materials in actuators, sensors, magnetic probes, phase inverters, rectifiers, modulators, and transducers in solid state microelectronics and microwave devices [2,3].

Spinel ferrite nanoparticles are being intensively investigated in recent years because of their remarkable electrical and magnetic properties and wide practical applications in information storage system, ferro-fluid technology, magnetiocaloric refrigeration and medical diagnosis [4]. Among the spinels, mixed Zn ferrites and especially Ni–Zn ferrites are widely used in applications like transformer cores, chokes, coils, noise filters recording heads etc. [5]. While Ni–Zn ferrite posses higher resistivity and saturation magnetization, cobalt ferrite possess high cubic magneto crystalline anisotropy and hence high coercivity. The high coercivity is driven by large anisotropy of the cobalt ions due to its important spin orbit coupling. It is ferromagnetic with a Curie temperature (T_c) around 520°C, [6] and shows a relatively large magnetic hysteresis which distinguishes it from rest of the spinels. The synthesis of ultra fine magnetic particles has been extensively investigated in recent years because of their potential applications in high density magnetic recording and magnetic fluids [7]. Among the current methods for synthesis of mixed ferrite the combustion reaction method stands out as an alternative and highly promising method for the synthesis of these ferrites [8]. Magnetic properties measured at room temperature by vibrating sample magnetometer (VSM) reveal an increase in saturation magnetization with increase in cobalt concentration [9].

II. Experimental:

The ferrites with general chemical formula $Cu_xCo_{1-x}Fe_{2-2y}Al_{2y}O_4$ (where x= 0.0, 0.2, 0.4, 0.6, 0.8, 1.0; y = 0.05, 0.15 and 0.25) were prepared by the standard ceramic technique using AR grade cobalt oxide, copper oxide, ferric oxide and aluminium oxide. The compositional weights of powders were mixed physically and blended in agate mortar in acetone medium. The final sintering process was carried at 1000^0C for 48 hours. The slow cooled samples were heated at rate of 80^0C per hour. The pellets and toroids were formed by using respective steel die. A universal testing machine as well as Archimedes's method was applied for

determining the physical properties of the samples. The formation of the cubic spinel structure of the samples prepared is confirmed by X- ray diffraction analysis. Initial permeability can be determined by i) Bridge method, ii) Resonance circuit method and iii) standing wave method. We adopted the resonance circuit method for the measurement. Initial permeability of $Cu_xCo_{1-x}Fe_{2-2y}Al_{2y}O_4$ ferrites is measured with temperature in the extent of 300 to 800 K applying an HP 4275 A LCR meter and HP 4992 ALF impedance analyzer. The furnace was temperature regulated with ± 2.5 Oc accuracy. The initial permeability at room temperature is the role of frequency was measured over 20 - 106 Hz. Initial permeability (μ_i) was determined from low field inductance measurings with toroidal cores of about 100 turns using the formula [Patil R.S. et al (**1991**)10] discussed elsewhere.

$L = 0.0046\ \mu_i N^2 h \log (d_2/d_1)$

III. Results and discussion:

The lattice constants 'a' and 'c' for all prepared samples are calculated by using prominent (311) XRD peak [11]. The calculated and observed values of inter planer distance (d) are found in good agreement with each other for all reflections. The particle size (D) for all the ferrite samples is calculated by Debye Scherer formula, ionic site radii (R_A, R_B) and ionic bond lengths (A-O, B-O) are calculated from the formulae given by Gadkari et.al [12]. From the calculations of lattice constants 'a' and 'c' for all the prepared ferrites; it is observed that c > a; tetragonality ratio (c/a) is found in the range of 1.03 to 1.07. This result is in good agreement with previous report [13-14].

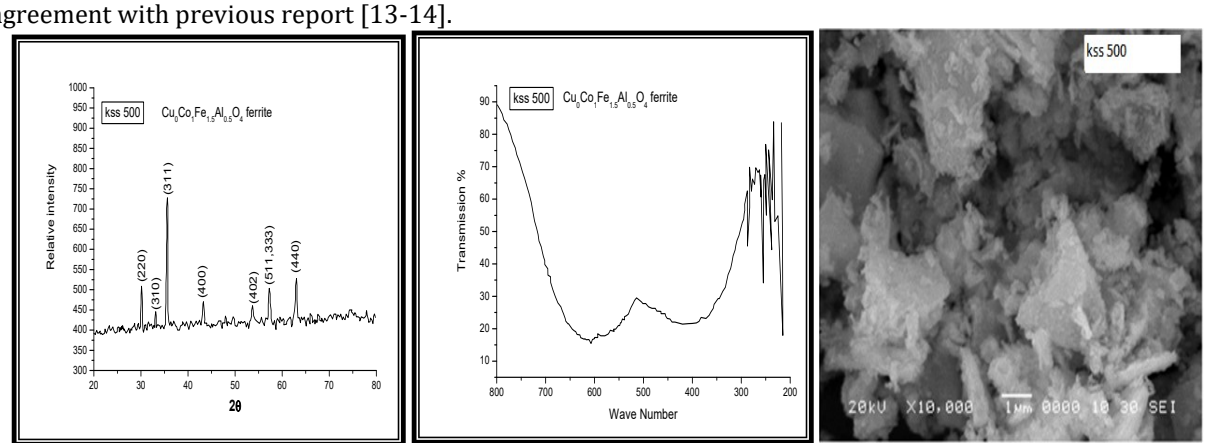

Fig: 1 Characterisation of ferrite samples by XRD,IR, SEM

The infrared absorption spectra showing two distinct absorption band ν_1 due to tetrahedral (A) site interstitial voids near 600 cm^{-1} and other ν_2 due to octahedral (B) site interstitials voids near 400 cm^{-1}. Our results in this present communication are well supported by previous reports [15, 16].

The close inspection of all micrographs revealed that there is continuous grain growth with well defined grain boundaries formed. The present system shows multi domain behaviour. No exaggerated grain growth is observed in any of this composition. The average grain size is found to decrease with increase in Al content in copper cobalt ferrite. However in the present system the grain growth shows generally an decreasing trend with aluminum content, which is rather expected because of multi-domain behavior of these compositions in copper cobalt ferrite. Grain growth is almost accompanied with grain size, which is increasing with copper and aluminum content. So it appears that copper and aluminum content favours the grain growth. The scanning electron micrographs shown below

The variation of initial permeability as a function of frequency (shown in fig.2) depicts the dispersion of initial permeability up to 1 KHz and beyond that it remains frequency independent. This low frequency dispersion in μ_i is attributed to domain wall displacement. The absence of low frequency resonance indicates that there is no domain wall motion. Thus, the low frequency dispersion in the ferrite compositions is only due to domain wall displacement. Initial permeability linearly decreases with increases the copper content as well as aluminium content.

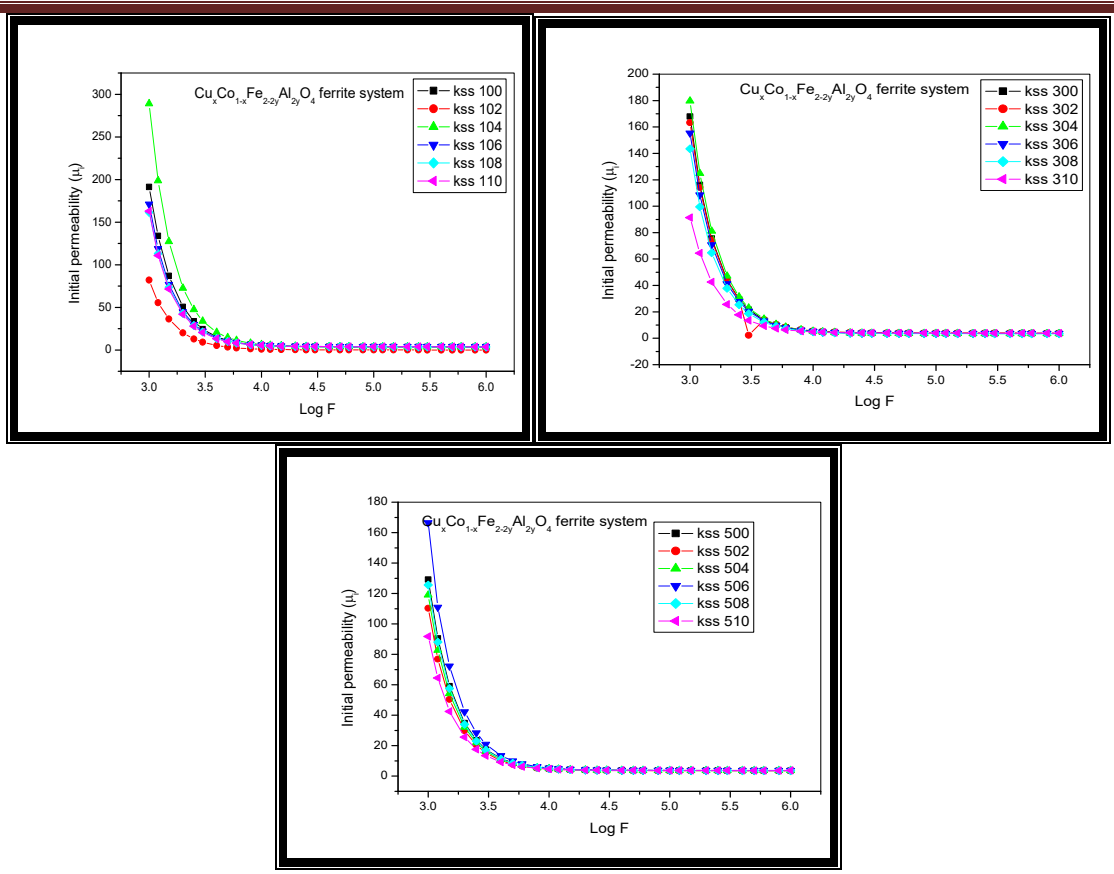

Fig 2: Variation of initial permeability with frequency for $Cu_xCo_{1-x}Fe_{2-2y}Al_{2y}O_4$ ferrite system

IV. CONCLUSION:

Copper cobalt ferrite is partially inverse spinel ferrite. Addition of Al^{3+} ions replaces Fe^{3+} on (B) site resulting in increase of lattice constant a, decrease in ionic radii(R_A) and bond length(O-A). The lattice constant obtained from XRD data shows increases. The initial permeability decreases with increase in frequency.

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Chemical bath deposition (CBD) of ZnO thin films in aqueous medium and its characterizations

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ABSTRACT: *Zinc oxide (ZnO) thin films have been deposited onto clean glass substrate using simple and cost-effective chemical bath deposition (CBD) method. The structural, morphological and chemical composition of the deposited films has been studied by X- Ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy techniques respectively. From XRD pattern polycrystalline nature of ZnO thin films was observed. The crystallite size of intensive (002) peak is 22.33 nm. The SEM images show denser layer ZnO nanoparticles. The EDX pattern show peaks of Zn and O, while other impurity peaks are not present.*

Keywords: *ZnO; thin films; CBD; XRD; SEM.*

I. Introduction

Nanocrystalline semiconducting materials have involved a great contract of attention because of their size reliant on properties and wide variety of applications. ZnO is one of the most remarkable semiconducting material.It has the band gap energy of 3.37 eV and is considered a potential material for use in gas sensors, solar cells, chemical sensors, piezoelectric transducers, photocatalyst, etc. [1]. As compared to other oxide material ZnO material is cheap and easily available material [2, 3].

Different methods have been used for synthesis of ZnO thin films such as spray pyrolysis, plasma-enhanced chemical vapor deposition, hydrothermal, sol-gel, chemical bath deposition, SILAR, reflux, co-precipitation, electrospinning, ultrasonic-assisted, etc. [4-8].In recent periods much interest has been created around the chemical bath deposition (CBD) method. The CBD method is simple, inexpensive, eco-friendly and appropriate for uniform deposition.

In present work, we have prepared ZnO thin films via CBD method. In the XRD patterns, no impurity peaks are observed. It means CBD method is suitable for ZnO thin films preparation. The SEM images show denser layer of ZnO material onto the substrates. The EDX pattern also show no other impurity peaks. Above all properties detects CBD is convenient technique for deposition of ZnO thin films.

II. Experimental

Preparation of ZnO thin films:

The glass was used as the substrates, which was cleaned with detergent liquid drop and chromic acid, followed by rising with double distilled water and finally treated with ultrasonic waves for 15 min and used for deposition. The source of zinc was 0.05 M zinc nitrate and to make it alkaline, aqueous ammonia was added. The pH of resultant solution was ~ 8. When the bath attained the temperature of 45 ^{o}C, the precipitation was started in the bath. The substrate coated with Zn (OH_2) thin films were removed after 100 min from the bath, washed with double-distilled water, dried in air. The deposited substrates were annealed in air for 380 ^{o}C.

III. Results and discussion

Structural studies:

Fig. 1 shows typical XRD pattern of annealed ZnO thin films deposited on glass substrate. The XRD patterns show polycrystalline nature of ZnO thin films. In XRD pattern five peaks of ZnO material is observed. The hkl planes for ZnO material is (1 0 0), (0 0 2), (1 0 1) and (1 0 3). ZnO films show high intensive peak at (002) plane. The XRD pattern of ZnO material is in good agreement with standard values of JCPDS card no. 80-0075 [9]. The crystallite size from Scherer's of intensive 002 peak is 22.33 nm.

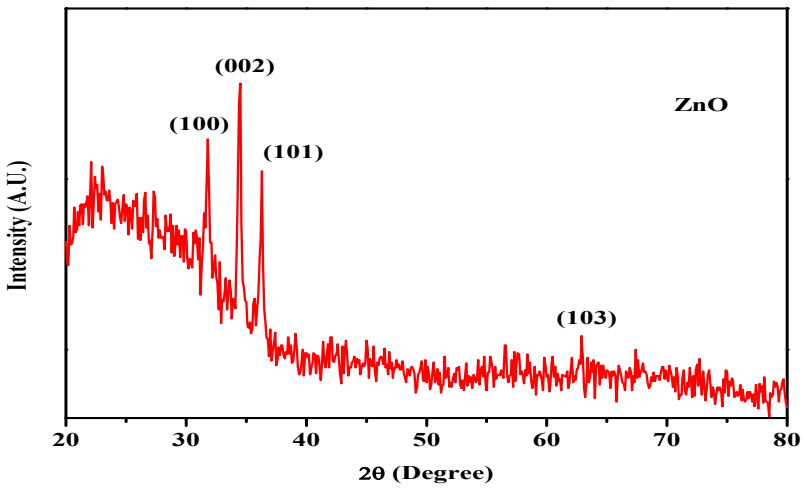

Fig.1. XRD pattern of ZnO thin films.

Surface morphology study:

The surface morphological studies of ZnO thin films have been carried out from scanning electron micrographs at three different magnifications. Fig. 2 shows the SEM images of ZnO thin films at magnifications (a) X 6000 and (b) X 30000 on glass substrates.From the micrograph, one can see that, the denser morphology of ZnO thin films on the substrates and small overgrowth particles are observed on the surface. It means CBD method is useful for denser layer formation of ZnO thin films. The SEM image observed in our study is different than others reported images of ZnO which is prepared by CBD method [6].

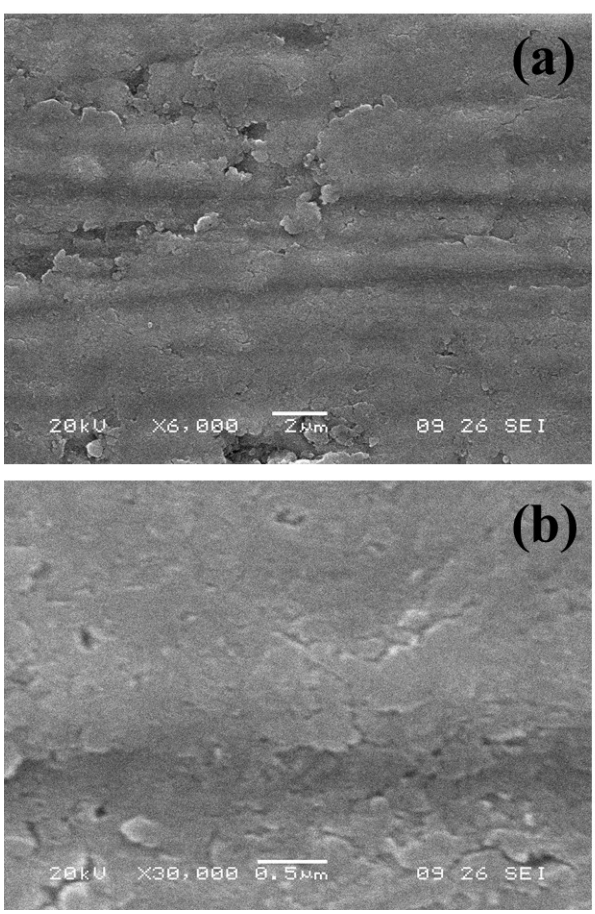

Fig.2. SEM micrographs of ZnO with different magnifications (a) X 6,000 and (b) X 30, 000.

Chemical composition study:

From energy dispersive X-ray spectroscopic (EDX) characterization we get the elemental analysis or chemical characterization of a given sample. EDX pattern shows the nearer composition of Zn and O. The EDX pattern of annealed ZnO thin films is as shown in Fig. 3.In the EDX pattern atomic and elemental wt. %. of Zn and O is determined. In the EDX pattern peaks of other materials is not observed. Due to this reason we confirm there is no impurity peaks in our samples. The samples ZnO show more intensive peaks of Zn and less intensive peaks of O. It means Zn is more than O in prepared ZnO thin films.

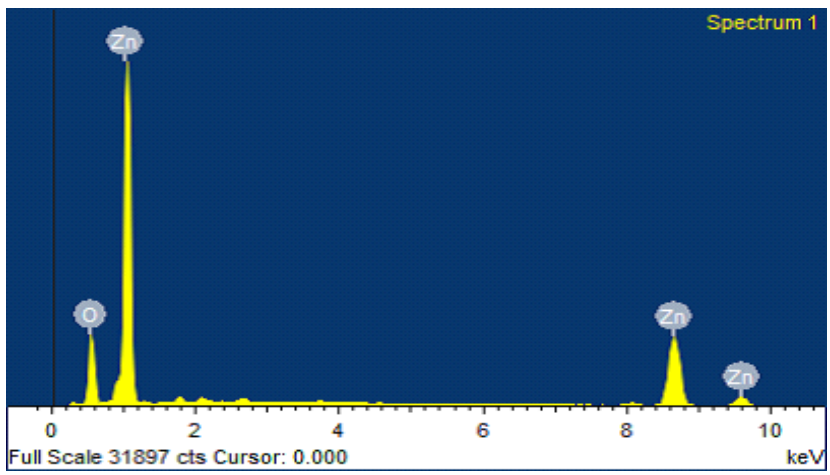

Fig. 3. EDX pattern of ZnO thin film.

IV. Conclusions:

The ZnO thin films have been synthesized via simpler and less time consuming CBD method. In the XRD pattern impurity peaks are absent. The XRD pattern also shows the polycrystalline nature of ZnO material. The crystallite size (D) value of more intensive peak isobserved to be 22.33 nm. SEM images display denser layer of ZnO martial for two different magnifications. The EDX pattern shows absence of impurity peaks which detects purity of ZnO. All above properties show ZnO material easily prepared by cost-effective CBD route. In feature it will be useful various applications.

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Studies On Consequence Of Temperature On Physical Properties Of CdSe Thin Films Synthesized Using Chemical Bath Deposition Method

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ABSTRACT: *Chemical bath deposition (CBD) method is the most easy, low-cost and appropriate method for synthesis of compound semiconductors. Present work discusses synthesis of cadmium selenide (CdSe) thin films on stainless steel (SS) and fluorine doped tin oxide coated glass substrates by a facile chemisynthesis route. Cadmium sulfate was used as source of cations while sodiumselenosulfate was used as source of anions. Various synthesis parameters are optimized so as to obtain the best photoactive deposit. The effect of deposition temperature on structural, optical and morphological properties was studied. Thin film samples deposited at various temperatures are characterized using X-ray diffraction, field emission scanning electron microscopy (FE-SEM) and contact angle measurement techniques. Structural study reveals presence of mixed crystal structure. Wettability study reveals increase in hydrophilic nature with increase in deposition temperature.*

Keywords: *CdSe, chemical bath deposition, temperature, Photoelectrochemical, FE-SEM, Wettability studies.*

1. Introduction

Since last few decades compound semiconductors of II-VI group have fascinated a great extent of attention because of their all-embracing applications [1, 2]. Among various semiconductors, Cadmium selenide (CdSe) is one of renowned II-VI group semiconductors, which has awestruck global researchers on account of appropriate properties. CdSe nanocrystals exhibit attractive properties like quantum size effect [3]. A suitable band gap energy of CdSe (Eg = 1.7eV) makes it more approving for various applications as laser diodes, light emitting diodes, optical sensing agents, photoelectrochemical solar cells, photodetectors, photoelectric applications etc [4-6]. Researchers employed number of deposition methods such as vacuum evaporation, successive ionic layer adsorption and reaction, spray pyrolysis, electrode position, pulse plating, chemical bath deposition to grow CdSe thin films [7-10]. Among various methods, chemical bath deposition (CBD) is one of the most suitable deposition methods with no requisite of sophisticated instrumentation. Chemical deposition is simple, economical method which is suitable for large area deposition. In CBD, deposition takes place when ionic product just goes above solubility product [11]. Various deposition parameters like precursor concentrations, deposition time, pH of solution, temperature etc strongly control growth rate of the deposition [12]. Temperature is one of the most important factors that affect growth of grains. One of the consequences of higher deposition temperature is increased crystal size in the thin film. Temperature of deposition bath plays vital role in deciding the physical properties of deposit [13]. Thus present investigation discusses the influence of bath temperature on physical properties of CdSe thin films.

2. Experimental details

2.1 Deposition of CdSe thin films

The CdSe thin films were deposited on the clean stainless steel substrates. As contaminated substrate results into nonuniform deposit thus all the substrates were cleaned by procedure reported elsewhere [9]. All the chemicals used were analytical reagent grade and used without any purification. Cadmium sulfate ($CdSO_4$) was used as sources of cation and sodium selenosulphate (Na_2SeSO_3) was used as anion. Further cadmium cations were complexed using 30 vol.% liquor ammonia. Preparation of Sodium selenosulphate solution was done by procedure reported elsewhere [14]. The preparative parameters were optimized with several trials and by well known photoelectrochemical method [15].

For chemical depositon of CdSe thin films, 10ml solution of 0.05 M $CdSO_4$ was taken into a beaker of 30ml capacity. Liquor ammonia was added drop by drop under constant stirring condition. Initially addition of ammonia to cadmium precursor solution results into formation of milky precipitate of cadmium hydroxide $Cd(OH)_2$, which completely dissolves subsequent to further addition of ammonia solution. Finally,

10 ml solution of 0.05 M Na_2SeSO_3 was poured to the same. Four substrates were kept slanted by 15-20^0 to wall of beaker. The pH of bath was maintained at 12. Temperature of bath was allowed to raise from room temperature to 65, 75 and 85^0 C. At bath temperatures more than 85^{0}C synthesis found impossible because of steaming of water. Deposition of CdSe material at room temperature was acquired after long time period from 3 to 4 days. After 8hs, deposited substrates were taken out, repeatedly diped in doubly distilled water, air dried and stored for further study.

2.2Characterizations

The structural properties of CdSe thin films are studied using Philips X-ray diffractometer PW-3710 with Cu Kα source (λ=1.54Å). The 2θ is varied range from 10^0 to100^0. Rame-Hart USA equipment equipped with a CCD camera was used in measurement of contact angle thus the study of solid –liquid interface was undertaken. The JEOLJSM 6360 unit was used for surface morphological study.

3. Results and discussion

Films deposited at 27^{0}C and 65^{0}C were found reddish in color, that found to be altered to brown and blackish brown, corresponding to bath temperatures, 75^{0}C and 85^{0}C, respectively as shown in fig. 1

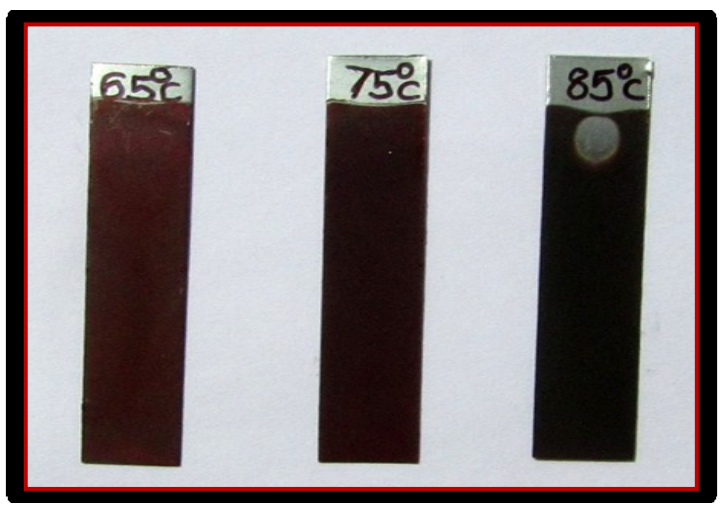

Fig. 1 Photograph of CdSe thin films deposited at different temperatures

3.1 Structural analysis

XRD patterns of chemically deposited CdSe thin films at different temperature values are shown in Fig. 2 The observed XRD patterns match well with Standard JCPDS data card no. 00-019-0191 ensuring the formation of CdSe with a metastable cubic (Sphalerite) crystal structure. The peaks designated by 'SS' corresponds to stainless steel which may be because of interference from XRD signals of the substrate.

The diffraction pattern of thin film deposited at temperature 65^{0}C (designated as (a)) shows only two diffraction peaks at 2θ = 25.5◦ and 50.6◦, corresponding to (111) and (311) planes of cubic phase respectively. The diffraction pattern for thin films deposited at temperature 75^{0}C (designated as (b)) observed to contain (220) plane, the along with (111) and (311) planes at 2θ values 42.4◦, 25.6◦, 50.6◦, respectively.

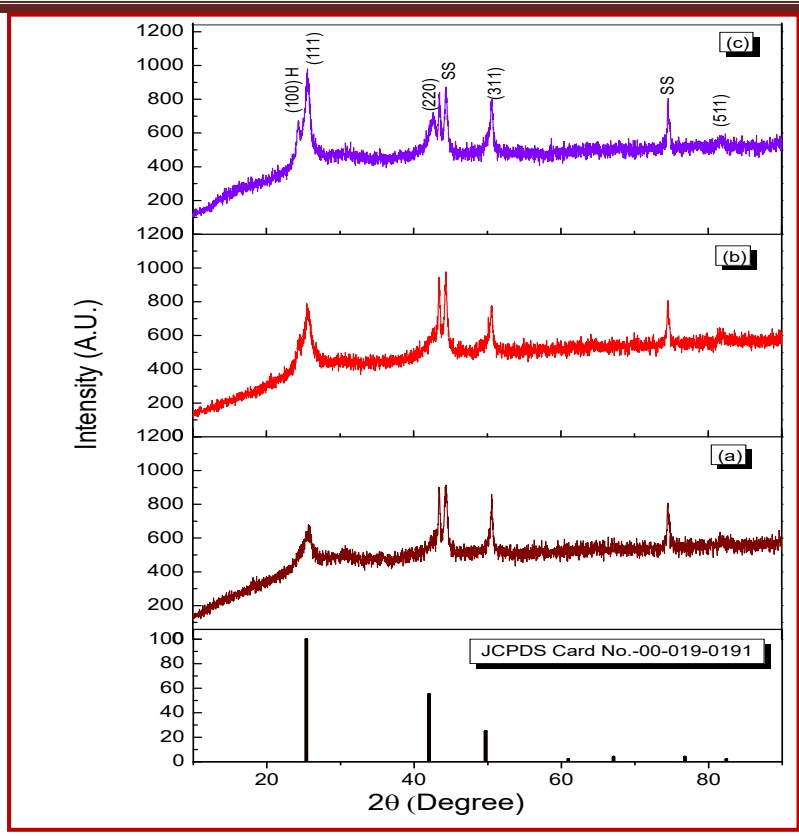

Figure 2: X-ray diffraction spectra of chemically deposited CdSe thin films at deposition temperature (a) 65 (b) 75 and (c) 85^0C.

The XRD pattern corresponding to deposition temperature 85^0 C (designated as (c)) reveals that the diffraction peaks become sharper with drop off in full width at half maximum showing enhancement in crystallinity and particle size in company with phase transformation. The diffraction pattern contains five diffraction peaks at 2θ values 24.3◦, 25.5◦, 42.7◦, 50.5◦ and 81.8◦. The diffraction peaks observed at 25.5◦, 42.7◦, 50.5◦ and 81.8◦ are indexed as (111), (220), (311) and (511) planes confirming formation of cubic phase which are in good agreement with earlier reports [16]. While the diffraction peak observed at 24.3◦ is indexed as (100) plane of wurtzite hexagonal phase [JCPDS data card no.00-002-0330] confirming phase change. Good enhancement in crystallinity is observed with increase in temperature. The observed values of interplaner spacing 'd' are found to be matches well with standard values of interplaner spacing 'd' signifying the formation of CdSe in thin film form. In all XRD patterns obtained at different temperature, a peak corresponding to the plane (111) is found to be prominent as compared to the other planes. Intensity of this plane increases with increase in temperature.

This can be explained as, since the rate of reaction mainly depends upon temperature. At lower deposition temperature, rate of reaction is slow, which increases with increase in temperature. Increase in bath temperature, increases thermal dissociation of cadmium cations from complex in conjunction with fast liberation of selenium anions from hydrolysis of Na_2SeSO_3. It guarantees high concentration of free cadmium cations and selenium anions in the deposition bath, which increases rate of reaction in combination with deposition rate. Consequently the crystallite size of CdSe thin film increase by way of deposition temperature. It shows that the temperature deposition bath has an effect on the crystal structure and crystallinity of the deposited thin film.

3.2 Wettability studies

Wettability study discusses the interaction between electrode with electrolyte which is described by value of contact angle.

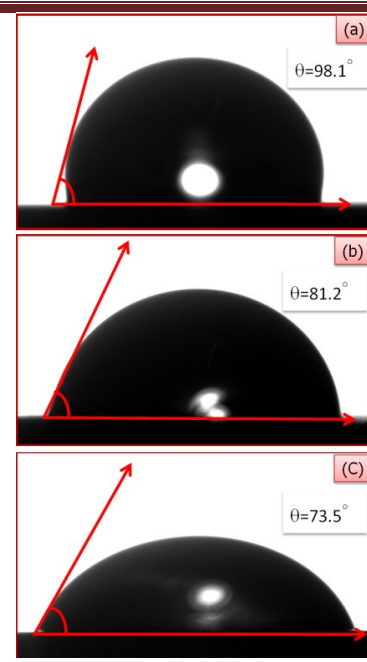

Figure 3. Contact angle measurement images of CdSe thin films deposited at different temperature values ((a), (b) , (c) corresponds deposition temperature 65, 75 and 85^{0}C)

Water contact angle measurement images for CdSe thin films deposited at different temperatures are shown in Fig.2.This decrease in water contact angle is attributed to increase in surface roughness. Smaller the contact angle more is the hydrophilic nature of electrode surface which results into intimate contact between photoanode and redox electrolyte in solar cell.

The water contact angle values found to be decreased from 98.1^0 to 73.5^0 with increase in temperature. Smaller be the contact angle more will be the hydrophilic nature of electrode surface [17].

3.3 Morphological studies

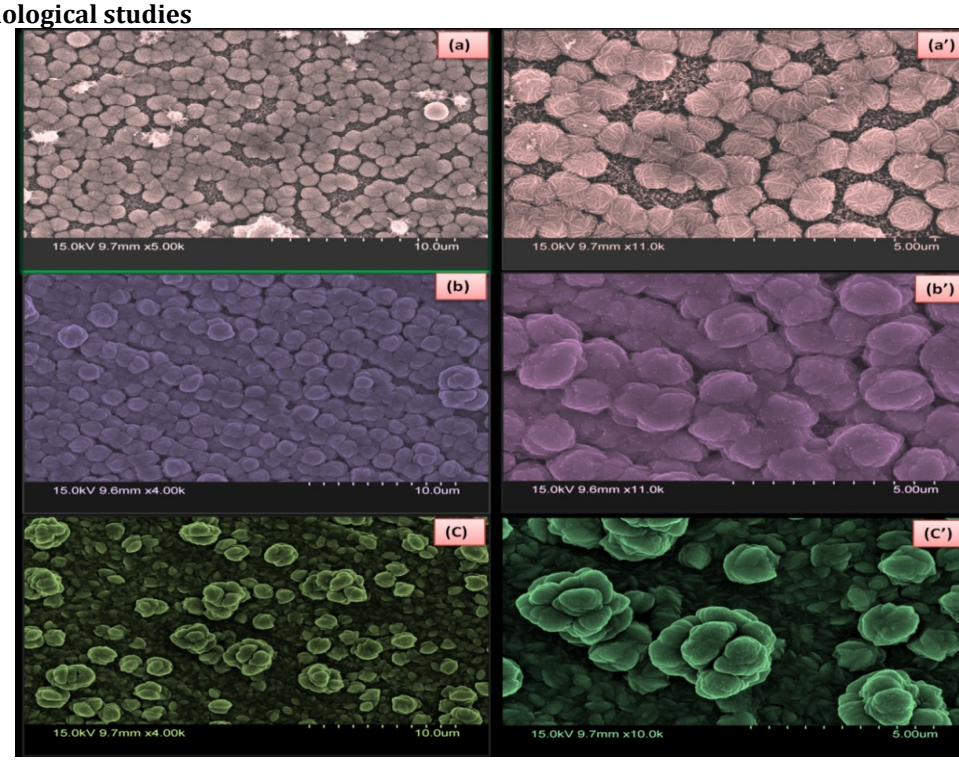

Figure 4: Lower and higher magnification FESEM images of CdSe thin films deposited at different bath temperatures [(a),(a')-65^{0}C, (b),(b') -75^{0}C and (c),(c')- 85^{0}C].

The surface morphology of CdSe thin films deposited at different temperature values was inspected using field emission scanning electron microscopy (FESEM). Figure 4 shows lower and higher magnification FESEM images of CdSe thin films. Fig. 4(a), (a') show lower and higher magnification FESEM images of CdSe thin films deposited at deposition temperature 65^0C. It shows pebble like morphology covered by fiber like structure on it. Pawar et.al [2] reported analogues morphology of chemically synthesized CdSe thin films on glass substrates. Films deposited at lower temperature are detachable. FESEM image (b), (b') of thin films deposited at temperature 75^0C shows more compact morphology. It shows fused pebbles like morphology. Compact structure with agglomerated grain like structure found to be deposited corresponding to temperature 85^0C. Well adherent and good quality films with cauliflower like morphology are found to be obtained corresponding to deposition temperature equal to85^0C. Surface morphology found to be significantly altered with the deposition temperature.

4. Conclusions

CdSe thin films are successfully synthesized by the chemical bath deposition method at different deposition temperatures. Structural studies reveal enhancement in crystallinity and particle size in company with phase transformation with increase in temperature from 65 to 85^0C. Wettability study shows, film deposited at temperature 85^0C has more hydrophilic nature with contact angle of 73.5◦. Surface morphology of CdSe thin films found to be significantly modulated via the parameter, bath temperature.

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Development of Breathalyzer using PIC Microcontroller

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ABSTRACT: *The intention behind to carry out this experiment is, to reduce the accidents caused in industries as well as on road by consumption of alcohol. The system becomes more helpful for reducing fatality ratio. The PIC16f877A microcontroller is interfaced with MQ-3 Sensor. BAC (Blood Alcohol Concentration) is displayed on 16x2 LCD display. The buzzer will make sound and the electromagnetic relay will shuts off the engine of vehicle / the machine on which suspect is working, if the BAC goes higher than normal limits.*

Keywords: *Fatality Ratio; PIC16F877A; MQ3; LCD; BAC; LED; Buzzer; Relay; Engine.*

I. Introduction:-

Alcohol is a major global contributing factor to death. In industries and road accidents alcohol seems to be mortal. Approximately, every 33 minutes there is one person in the wide world, who dies at one traffic accident caused by driving under the influence of alcohol [6]. This becomes very serious public health problem. The accidents happened during drunk and work cannot be neglected. The problem is unrecognized and hidden due to lack of good quality research data. In India, many road accidents was occurred due to the drunken driving, so there are several acts to punish the drive person during the road accidents. Nowadays the policeman checks to the driver manually, but it is very difficult to check each and every driver, so they could not avoid the road accidents. This system measures the amount of alcohol in air, which is air come from driver's mouth. To determine the percentage of Blood alcohol content by the MQ-3 sensor and it is converting into electric signal for display the percentage of alcohol. Further this system alert the persons by buzzer

After drinking, the driving judgment power of the driver gets disturbed which is a danger to road safety. The standard limit to the concentration of alcohol in air is 0.03% in India. Alcohol consumption under no control and monitoring increases the number of industrial accidents and the traffic accidents victims. Methods to test and record the alcohol level -BAC (blood alcohol concentration) in the body can reduce the risks of traffic accidents.[5].

II. Methodology

The concentration of alcohol is sensed by a very sensitive MQ-3 sensor. The sensor shows higher sensitivity to alcohol in air. The MQ-3 gives the output voltage in positive proportion to the concentration of alcohol. The output voltage from MQ-3 is given to the ADC of microcontroller for further digitized processing. Microchip made 8-bit PIC (Peripheral Interface Controller) microcontroller is used in this experiment with 4 Mega Hertz as its operating frequency. On chip ADC with the 1023 bits resolution, provided by microcontroller. The block Diagram of experiment is as shown in Fig (1).

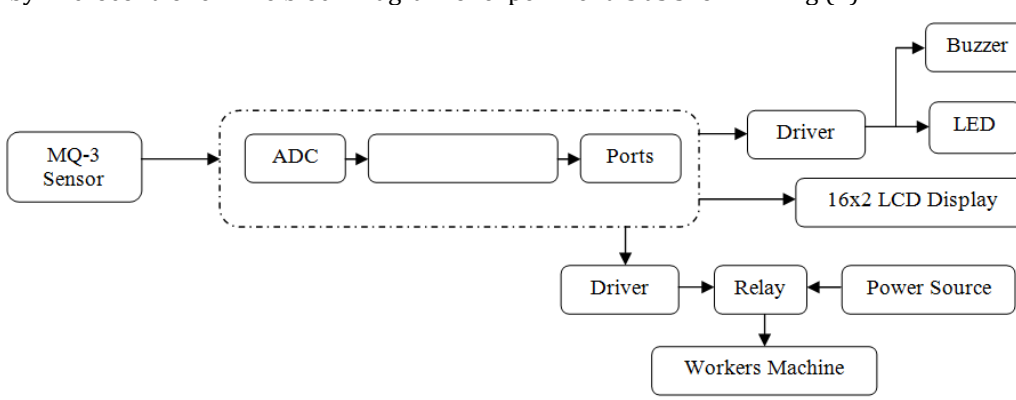

Fig-1 Block Diagram of System

The 16x2 LCD Display is interfaced with Microcontroller in 4Bit low programming mode, to Port B. as shown in Table (1)

Microcontroller Port Pin	LCD Pin
RB0 to RB3	D7 to D4
RB4	RS
RB5	EN

Table -1 Port pin assignment structure

The Red LED and Buzzer is used to alert person to know about the BAC. They will notify when BAC is above the normal value. The port pin RD.1 and RD.2 are configured for controlling buzzer and relay. A Simple transistor (BC 547) based drive is used for LED and Buzzer.

The Program is written in Embedded C and compiled in MPLAB X IDE void Initialize () &LCD_init () function is used for initialization of all peripherals attached with microcontroller and to calibrate LCD, LCD_init () function is used. MQ_3_read () function is used for reading analog voltage from alcohol detector.

Concentration of Alcoholin Breath (grams/liter)	BAC (%)
0	0.00%
9.5×10^{-6}	0.02% (maximum legal limit in India is 0.03%)
1.9×10^{-5}	0.04%
2.8×10^{-5}	0.06%
3.8×10^{-5}	0.08%
4.7×10^{-5}	0.10%
5.7×10^{-5}	0.12%
9.5×10^{-5}	0.20%

Table-2 Concentration of alcohol in breath and their corresponding BAC value

In India the maximum legal limit of BAC is 0.03%. When the percentage of BAC goes above 0.03% buzzer starts sounding, LED will glow. Thereafter relay will shut off the power supply going towards machine.

I.FLOWCHART:

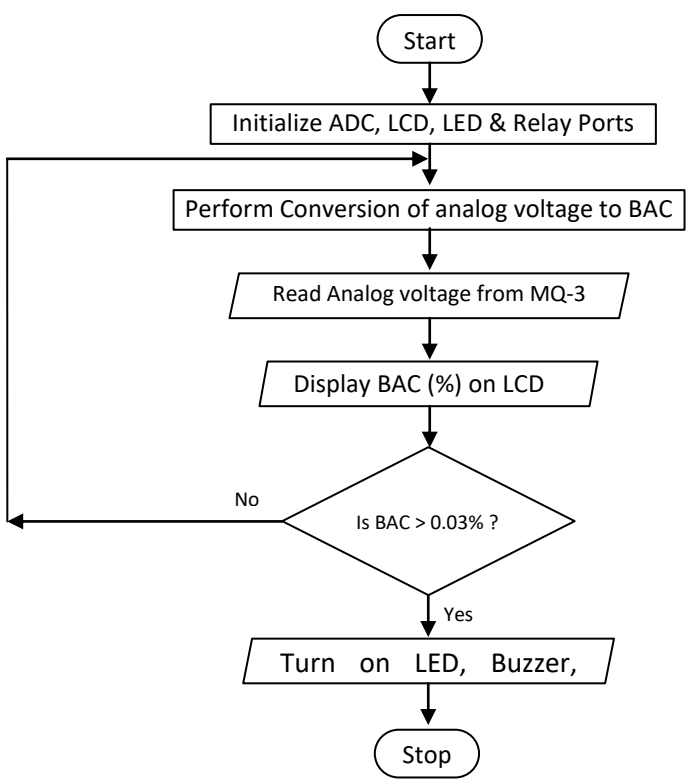

Fig-2:- Actual Implemented System

III. Results

The table shown below gives the specifications of MQ-3 Sensor. When the system is switched on, the sensor takes time to stabilize its readings.

Parameter	**Values**
Time taken for sensor to stabilize	15 to 20 seconds
Operating temperature	Room temperature = 29°C
Heater temperature	45°C to 60°C
Input voltage	5V
Output voltage range	0 to 4.75V
Saturated output voltage value	4.75V
Normal atmosphere detected value	1.85V to 2.5V
Human's breath without alcohol detected value	0.50V to 1.84V

Table 3: Specification of the sensor

The conversion of the sensor output for different alcohol concentration and its corresponding BAC(%) values are as in the table 4.

Voltage(V)	**BAC (%)**
0	0.00
4.02	0.02
4.14	0.04
4.25	0.06
4.32	0.08
4.42	0.10
4.75	0.20

Table 4: Conversion of MQ-3 output voltage corresponding BAC (%) values

CONCLUSION

Use of PIC makes the system very flexible. With the help of system we can reduce the industrial and road accidents happening due to alcohol consumption. It is easy to send data towards the other systems like GPS, GSM etc. because of microcontroller based system.

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LOW COST SOLAR POWERED EGG INCUBATION SYSTEM

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ABSTRACT: *In rural area of India, animal husbandry is one of the agro supportive and complementary business. The backyard poultry farming is one of that. It increases employability of small scale farmers as well provides some additional income. With regard to this we have experimented a low cost solar power egg incubator. This system takes power form solar panels in day time simultaneously it stores it into battery and utilizes at night time. The system virtually creates suitable environment for eggs hatching without hen. The PIC-16F877A microcontroller controls the temperature which is sensed by temperature sensor LM-35. For providing heat inside the incubator, Simple heating coil mechanism is used. The 16x2 LCD is used to display inner incubator temperature.*

Keywords: *animal husbandry; poultry; farmers; solar power; incubator; hatching; PIC;LM-35; LCD.*

I. Introduction

An egg incubator is a device which provides a virtual environment for production of chicks, without hen. In rural part of India, farmers are using traditional method for hatching eggs. This method have some drawbacks, such as farmer needs a broody (non-egg laying) hen to hatch eggs. Hen can incubate 12-15 eggs by sitting on eggs. If broody hen is used for hatching eggs, the farmer has a daily task to feed hen. This traditional process takes too much of time and it is non profitable for farmers.

Hatching eggs deteriorate with storage and should not normally be kept for longer than seven days before being set for incubation[1].The small incubator will be helpful for increase hatchability of eggs. Which then improves production of chicks and eggs for protein requirement of large population. This also leads to increase the economic condition of rural farmers. The credit of incubator goes to Chinese and the Egyptians for inventing artificial incubation techniques [2].

The low cost solar powered egg incubation system creates virtual environment by maintaining the temperature at desirable level. During day time, the heat required for incubation is generated through electricity from solar panels with help of electric coil. Simultaneously battery is charging from solar panels. After dusk, power from solar is inadequate to produce required amount of heat, then battery is utilized up to next dawn. For this experiment temperature is kept at 38.50C full incubation period. Intention of doing this experiment is, to develop cost efficient incubator affordable for every rural farmer. With less investment to increase their earnings.This system consist PIC 16F77A microcontroller as the brain of the circuit.

II. Block Diagram

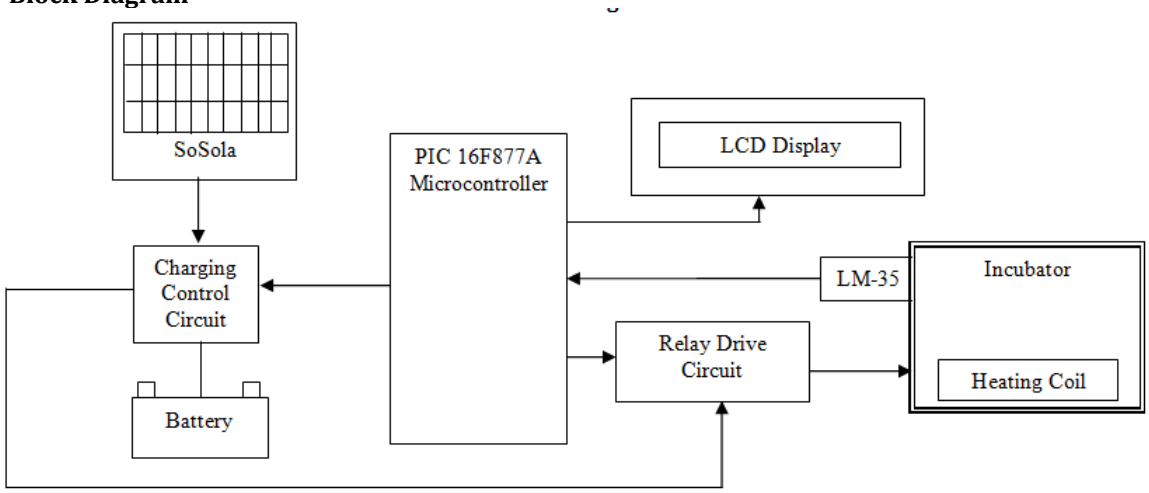


Figure 1. Block Diagram of Incubator

III. Materials and Method

The system is designed around Microchip made PIC16F877A Microcontroller. Entire block diagram of system is as shown in **Fig.1.** Solar panel generates DC output voltage. This voltage is utilized by two ways, first is to feed heat coil and second is to charge battery. Charging control block controls overcharging of battery. When solar power is not available, charging control block switches to battery supply to feed heat coil.LCD Display continuously displays the incubator temperature in ^{0}C. Relay drive circuit shuts OFF and ON power of heating coil as per instructions of Microcontroller, when temperature goes above and below 38.5^{0}C.

Temperature Sensor

Temperature sensor used in the system is LM35, a precision semiconductor integrated circuit sensor.Its output is linearly proportional to Celsius temperature. The benefits of LM35 include, i) it gives linear output of 10mV per degree centigrade, ii) It doesn't require any externalcalibration, iii) Good ensured accuracy of 0.5^{0}C as measuredat 25^{0}C iv) very low Nonlinearity only ±¼°C, v) very lowpower dissipation, only 60µA has been drawn from thesupply, vi) very low self-heating of less than 0.1 °C in stillair, because of low power dissipation. The Precision LM-temperature sensor LM-35[3] is used to sense temperature inside incubator.

This analog output of temperature sensor is connected to On-chip ADC of PIC Microcontroller, then this analog value is converted with software algorithm into ^{0}C. The Variants of LM35 series are as shown in table 1[5].

Sensor	Temperature Range	Output
LM35A	-550C to +150 0C	10mV/0C
LM35CA	-400C to +110 0C	10mV/0C
LM35D	00C to +100 0C	10mV/0C

Table 1. LM35 variants

Microcontroller

The PIC 16F877A Microcontroller have 8 channels of ADC with 10 bit resolution[4].The temperature range considered for the application is 0^{0}C to +50^{0}C and hence corresponding output voltage of sensor will be 0V to 50*10mV=500 mV i.e. 0.5V. The reference voltage of 2.56V with Step size of 2.5mV is suitable for the application. With these settings the system will be capable of displaying temperature between 0 to 50^{0}C, with resolution of 0.25^{0}C.

The microcontroller have sufficient no. of I/O pins for interfacing LCD display and for relay drive circuit.

Solar Panel

The Solar Panel is selected so as it gives sufficient power to heating coil and battery for charging. During heating the coil requires 800mA current. For other circuit operations current is considered about 200mA. i.e. total current requires is near about 1Ampere from solar panel. The solar panel used in this system is 20 watt solar Panel. Have open circuit Voltage V_{oc}= 21.7 Volts and short circuit current Isc = 1.25 Amp this is sufficient for system to normally work.

Battery

Battery is used during night time to power up heating coil and motherboard. The total current requires is near about 1Amp. The heating coil doesn't requires continuous current, it needs current when temperature inside incubator goes below 38.5 ^{0}C. Therefore we have selected 10Ah battery. If continuous current of 1 amp is drawn from battery it will give power about 10 hours. As stated above we don't need continuous current, this battery is sufficient to drive all the loads during night time.

Relay drive Circuit

Microcontroller pins are not capable to drive the relay directly. So we need a driver circuit. Here, Relay drive circuit consist of a transistorized switch to drive relay. The BC-547 transistor based drive is used. The relay with operating voltage of 12 volt and current handling capacity of 5Amp is used.

The whole system is implemented as shown in **fig.2**

Software Description

The software code of this system is developed in embedded-C language and compiled in Micro-C compiler for PIC microcontroller. The fig.3 shows flowchart of microcontroller's program. Microcontroller initializes its memory and peripheral devices. Firstly it checks whether the power is coming or not from solar panel. If power is coming, it checks the battery voltage by reading ADC. After reading ADC value from

battery, it will convert it into battery voltage. When battery is sufficiently charged, microcontroller sends signal to charging control circuit for stop charging of battery and avoid overcharging. If no power is coming from solar panel, microcontroller sends signal to charging control circuit to drive the heater coil inside incubator.

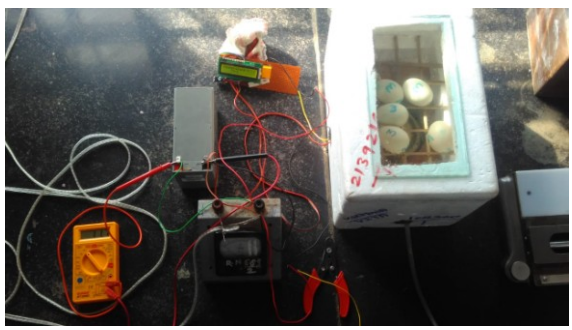
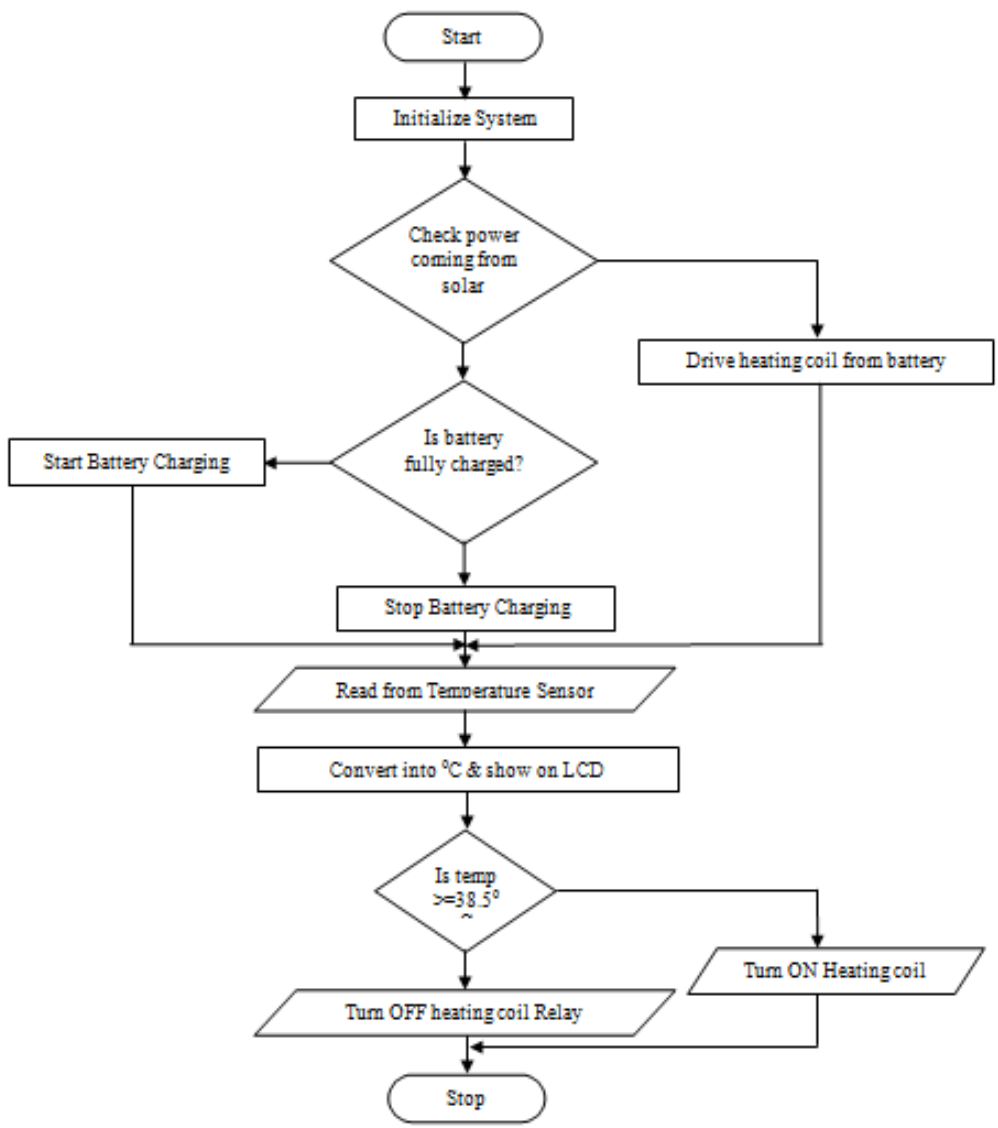


Figure 2. Actual system

Now microcontroller reads Analog data from LM-35, then convert it into ^{0}C, shows temperature on LCD. If temperature is below 38.5^{0}C, sends signal to relay drive circuit to start heating. When temperature goes above 38.5^{0}C, microcontroller sends signal to stop heating.

Flowchart:



IV. Results and discussion –

During the tenure of this experiment we found that, the solar powered incubator is economically helpful for the farmers. It will fulfill the protein requirement of fast growing community. System reduces need of broody hen. The figure shows hatched eggs tested in zoology lab of our college.

The rural farmers get lifetime income from the system, by one time investment. The system works without electricity i.e. it uses solar power which overcomes the power failure effects inside the incubators which are working on electricity. The system will become a good example of utilization of renewable energy.

Incubating eggs were tested for system working at 5th and 7th day of incubation. Following **fig.4** shows the tested results.

Figure 4. Incubated Eggs

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REMOTE PHYSIOLOGICAL PARAMETER MONITORING SYSTEM FOR CATTLE

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ABSTRACT: *The purpose for the development of this system is, to measure the Surface Body Temperature (SBT) of cattle. The system will become helpful to identify the illness, heat stress of the cattle by sensing the surface body temperature. System consists of two parts transmitter and receiver. The wearable belt on body of cattle works as transmitter. The Atmega-328p microcontroller is interfaced with LM-35 temperature sensor; these components are connected on a wearable belt. For the sake of wireless communication, the RF (Radio Frequency) transceiver module nRF24L01 is used. At the receiver section the same RF module is used for wireless data reception. With the help of 16x2 LCD (Liquid Crystal Display) the temperature data is displayed in the degree Celsius (^{0}C). If the surface body temperature of cattle goes above than normal, the buzzer will make sound.*

Keywords: *physiological; cattle; surface body temperature; wearable belt; Atmega-328p; nRF24L01 transceiver.*

I. INTRODUCTION

Surface Body temperature is the most important physiological parameter in animals. It is related to health, productivity and reproductive success. In animals surface body temperature increases because of illness, heat stress, toxin exposure, injury, or other health-related issues. Raised body temperature has been used to identify sick and heat-stressed animals. Rectal temperature is among the most commonly used measurement of body temperature [1].The normal body temperature is different in different types of animals. There are number of ways by which animals control the temperature of the body as Hair, wool, walking, running, shivering and the burning of energy in feed, keep the body warm [2]. Dripping, puffing, stumbling in mud, and lying in the shade cool the body.

The Surface Body Temperature of cattle varies regularly with both the reproductive cycle and disease status. Designing an automatic method for monitoring Surface body temperature may facilitate better management of reproduction and disease control in cattle. Here, we developed Remote Physiological Parameter Monitoring System (RPPMS) for cattle's Surface Body Temperature (SBT) to measure the temperature of metatarsus by attaching a special wearable belt designed to fit the anatomy of cattle's hind leg. Using this system, the Surface Body Temperature (SBT) on the metatarsus of the hind leg was successively measured during 24 hours a day with an interval of 4 hours.

As an important sign of both the health and physiological status of cattle, body temperature has been used to evaluate the regularity of oestrus and ovulation, pregnancy, parturition and disease occurrence in cattle [2].Measurement of Surface Body Temperature manually is costly and time-consuming also it increases the possibility of disease transmission. It is difficult to measure temperature over the course of a full day cycle as well. Therefore to establish a simple, chipper, efficient and automated method for cattle surface body temperature detection is needed.

The objective of this system is to establish a technology to automatically measure the SBT of cattle. We designed a specific device according to the anatomy of cattle's hind leg, using which the SBT on the metatarsus was successfully detected. The establishment of our Remote Physiological Parameter Monitoring System (RPPMS) for cattle's Surface Body Temperature based on LM-35 sensor (Fig.1) provides a highly reliable and automatic cattle surface body temperature detection system.

II. METHODOLOGY

In this experiment, we used commercially available LM-35 temperature sensor as shown in fig 1.The LM35 precision integrated-circuit temperature sensor whose output voltage is linearly proportional to the Celsius (Centigrade) temperature. It is integrated circuit that can be used to measure temperature with electrical output. It is more accurate and its operating temperature range is from -55 to 150 degree (^{0}C). For detecting SBT of the cattle, the range provided by LM-35 is sufficient. The Surface Body Temperature (SBT) was measured using our system from the metatarsus of the hind leg with sparse hair.

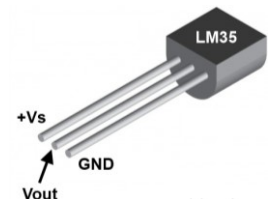

Figure.1 LM -35 Temp Sensor

The temperature sensor is mounted on the metatarsus with the help of wearable belt, where the muscle and blood vessels are rich. A sphygmomanometer type belt was designed to fix the temperature sensor appropriate to the cattle anatomy. The temperature sensor was placed in close contact with the muscle via the belt and other parts are mounted on other side of belt. This design minimizes the influence of environment factors like wind, leading to more accurate temperature measurement.

The Table 1 below shows the Normal range of SBT (Surface Body Temperature) in animals [3].

Table 1 SBT(Surface Body Temperature) in animals

Animal name	Normal Body temperature in °C
Cattle	38.5
Calf	39.5
Buffalo	38.2
Goat	39.5
Horse	38.0
Donkey	38.2

BLOCK DIAGRAM

The block Diagram of experiment is as shown in Fig (2).

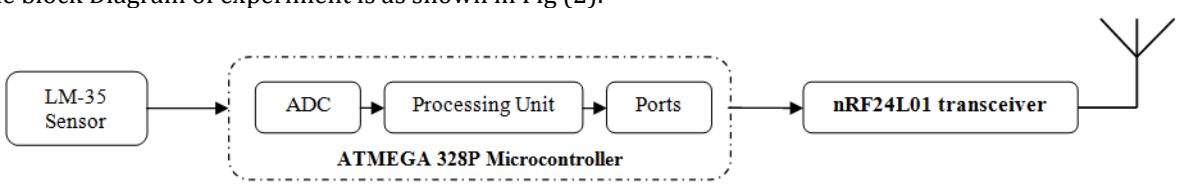

FIGURE-2.1 BLOCK DIAGRAM OF TRANSMITTER SECTION

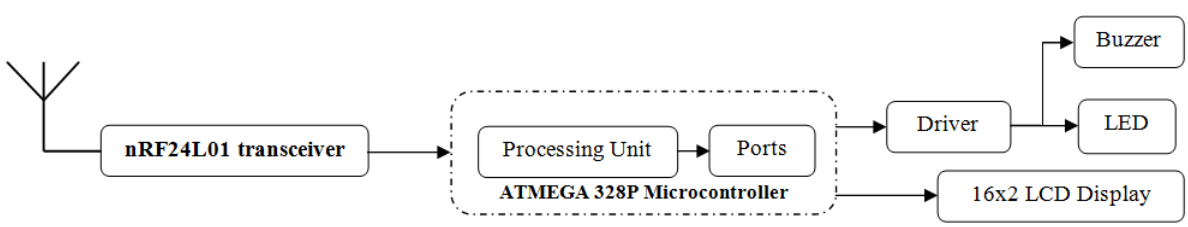

FIGURE-2.2 BLOCK DIAGRAM OF RECEIVER SECTION

III. Working of the system:

The transmitter section having temperature sensor LM-35 senses Surface Body Temperature (SBT) of cattle and gives analog output in voltage form and this analog voltage is given to 10-bit Analog-to-Digital convertor (ADC) of ATMEGA 328P microcontroller which is 8-bit AVR RISC-based microcontroller and operates between 1.8-5.5 volts. The 10-bit Analog-to-Digital convertor (ADC) converts analog voltage into digital output form and given to processing unit. The output of processing unit is given to nRF24L01 transceiver having 2.4-2.5 GHz ISM frequency band. This wireless data is received by receiver section of nRF24L01 transceiver and given to microcontroller ATMEGA 328P and displayed by using LCD.

The Buzzer is used to alert person to know about the SBT of the cattle. When Surface Body Temperature (SBT) of the cattle is above the normal value i.e. 38.5°C buzzer will sound. A Simple transistor (BC 547) based driver is used for Buzzer

IV. Result and Conclusion:

With the help of this system we can developed an automatic method for monitoring Surface Body Temperature may facilitate better management of reproduction and disease control in cattle. The system

provides a highly reliable and automatic cattle Surface Body Temperature detection system. It is easy to send information towards the other systems like GPS, GSM etc. because of microcontroller based system.

Following photographs shows actual implementation system. Belt is connected to hind limb of cattle with transmitter, right hand figure shows SBT on digital display.

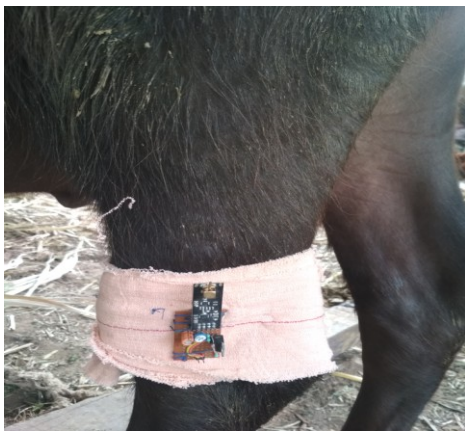

Fig. Actual System (Transmitter)

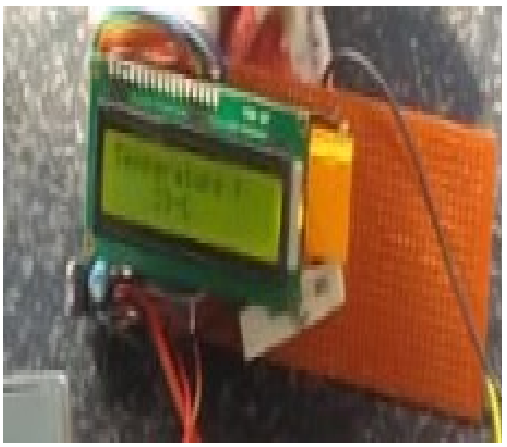

Fig 4.Receiver Section

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SCHEDULING OF CROP IRRIGATION AND FERTILIZER USING PIC

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ABSTRACT: *The electronic irrigation system provides water to the crop as per the moisture level of soil. In these types of systems pumps stops immediately when water reaches to moisture sensor. But to improve the quality of irrigation and crop the duration of watering should be greater; as well crop needs fertilizers as per environmental conditions. The work carried out in this paper is, the system developed for watering farm with the predefined schedule. Watering was done for predefined time period, as per data entered by user into android app; analyze this data and system follow the schedule of crop irrigation. In this system, sensors are used to check temperature and soil condition; PIC microcontroller compares these parameters, send to Smartphone controls the operation of irrigation system. For wireless operation, Bluetooth and for front end, the android application was used. To deliver fertilizers up to crop electronic valves and motors were used.*

Keywords: *Irrigation system, crop, watering, PIC, sensor, Smartphone, Bluetooth, android, fertilizers, valves, motors.*

I. INTRODUCTION

Indian economy is mainly based on agriculture, according to economic survey of India, agriculture contributes about 16% oftotal GDP (Gross Domestic Product) and in this contribution developed states are Punjab, Uttar-Pradesh, Madhya Pradesh, Haryana, Bihar, Andhra Pradesh, Maharashtra, and West Bengal. Agriculture is the enormous source of food production which reduces gradually increasing demand of human population [1]. Agriculture is not possible without irrigation, water used for the irrigation and fertilizer to increasing productivity. Irrigation is a manipulation of water to the crops at required time intervals where natural rainfall insufficient to support crops. Indian irrigation infrastructure includes major and minor channels from rivers, groundwater well-based systems but it require high man power; due to its low cost channel system widely used in farming. In which pipes are connected to electric pump, while pump started water flow through pipe from well, river to irrigate the farm. But the farmer fully engaged to irrigate the crop field with number of workers, large amount of water waste [1].

Ancient agriculture in India depends on climatic condition; rainfall depends on monsoons, uncertain annual average rainfall of about 1200 mm; the improvements in irrigation to improve food security reduce deficiencies in rainfall. The agriculture irrigation plays an important role that provides water and influences crop production. About 90 % of farmer use the traditional irrigation methods involves gravity flow of water for farms. Major amount of water wastes due to unplanned use of water [2]. Because the fields contain different plants such as trees, grass and vegetables; each field should be irrigated in a different plan having different period. As much as 50% use of water is wasted due to overwatering caused by inefficiencies in traditional irrigation systems. Scheduled irrigation systems supply the water to crops only for a specific duration and avoid overwatering to improveoutdoor water use efficiency. This modern irrigation method provides facility to apply the fertilizer with irrigation water.

The traditional irrigation as ditch irrigation, terraced irrigation, drip or sprinkler irrigation that controls manually to irrigate and fertilize land at irregular intervals [4]. Indian farmersvisit their agriculture fields periodically to check soil dryness level and start motor to irrigate respective fields [5]. It requires more water or due to overwatering amount of water waste and decrease the yield [6]. This problem can be rectified by adopting scheduled crop irrigation and fertilizer system using PIC microcontroller which linked with sensors through android app smartphone. The proposed system is used in drip irrigation which is better than other irrigation because it supply water and fertilizer slowly to the root zone of crop [7].

This irrigation system check the weather condition or soil content with help of moisture or humidity sensors, sends information to the farmer's smartphone through android app using PIC controller to turn on/off the motor and fertilizer valve of drip irrigation system. Resulting to provide required level of water for crops also avoid wastage of water, need of man power, save energy or money will increase the yield. The PIC16F777A controller based scheduling smart irrigation system will be most appropriate.

II. METHODOLOGY

The backbones of this experiment are moisture sensor, humidity sensor and FCTE45 heat sensor. The

sensor detects and measures the physical quantity as soil moisture and temperature from field, converts it into an electrical signal in the form of voltage or current is the output of sensors [7]. Temperature and moisture are inter-related parameter, the soil temperature increases, soil moisture decreases. The comparison of these both parameters can be used for the better yield of crops. The actual system design shown in fig.1

Fig 1. Actual System

A. Moisture Sensor

In agriculture irrigation system like drip irrigation need accurate moisture content for crops because the moisture in the soil plays an important role in the proper growth of crop. So, the moisture sensor is used in proposed irrigation system. The soil moisture sensors measure the volumetric water content (VWC) in soil, indirectly by using electrical property of soil, such as electrical resistance or dielectric constant [8]. The resistivity of soil decrease with increasing amount of water in soil moisture that means amount of water maximum the VWC count and conductivity of soil high [9].

The SW10 is a three terminal soil moisture sensor shown in fig 1; it is buried 4 inch to 6 inch deep in field. Basically soil is nonconductive by nature but presence of water in soil increases its conductivity, because conduction ions present in water. If the soil conductivity high its resistivity low then the electric current is high, soil resistance is varies from the 0 kilo-Ohms to 5 kilo-Ohms [7].

Fig 2. SW10 Soil Moisture sensor

B. Pump Dry Running Sensor

If no water in well, there no load on electric motor and motor run at high speed and draws the heavy current. Due to increased friction between rotor and stator, rise the internal temperature at the stator then motor operate in dry running mode.In this condition motor may be burnout, to prevent the motor damage by attaching the FCTE45 heat sensor device (shown in fig 3) to the outlet pipe of motor. This sensor senses the rapidly rising temperature at the outlet pipe of motor and sent to the PIC controller, which can stops the motor immediately.

Fig 3 Temperature sensor (LM35)

Table: Specification of FCTE45 heat sensor

Set Temp.	**45^{0}C**
Switching Voltage Max.	**250V**
Switching current Max.	**16 A**
Pipe diameter range	**20-220mm**

C. Humidity Sensor

Humidity is a present amount of water in air, humidity sensors measures the water vapor present in air. According to the measurement unit sensors divided into two types relative humidity and absolute humidity. Basically the relative humidity is measured by using relative humidity sensor. The electronic types of humidity sensors are resistive, capacitive and thermal sensors. It can also measure the moisture and temperature in the air. The DHT11 humidity sensor shown in fig 4, it has four terminals, two for power supply and two for humidity (DATA) and temperature (NC). In this irrigation system, the DATA terminal and GND terminal used for humidity measurement. The moisture substrate exists between these two electrodes (DATA and GND) and the resistance between them changes in accordance with humidity change.

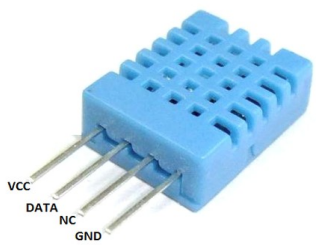

Fig 4 DHT11-humidity sensor

Resistive sensors consist of noble metal electrodes deposited on a substrate by photo-resist techniques. The sensor captivate the water vapor and ionic functional groups are dissociated, resulting in an increase in electrical conductivity.

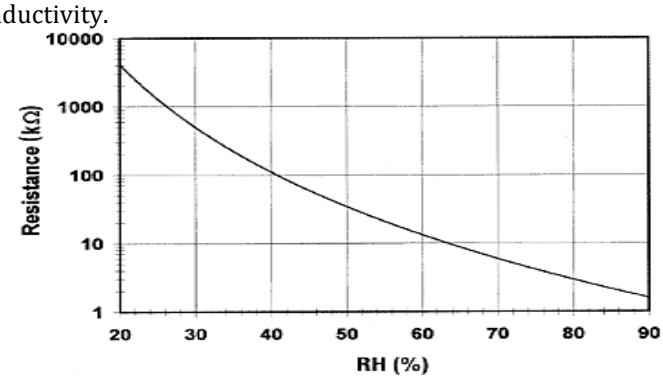

Fig 5. Humidity vs. Resistance curve of the resistive humidity sensor

The change in impedance or resistance of sensor is inversely proportional to humidity change. The exponential response curve of resistive humidity sensor is plotted for relative humidity in percent and resistance in kΩ at 25^{0}C temperature shown in fig5

D. Bluetooth Module

A Bluetooth technology is a high speed low powered wireless technology link, which is designed to connect smartphone. The IEEE 802.15.1 standardized protocol has derived a wireless personal area network based on the Bluetooth. A Bluetooth is uses serial port (USART) communication to communicate with microcontroller to exchange data over short distances. In future wireless high level Zigbee2MW module is used for long distance.

This module is suitable where wireless data transmission is needed in a master or slave mode. The board of HC-05 Bluetooth module is designed for wireless communicationover a range of up to 9 meters (30 ft) and it has 6 pins shown in fig 6.

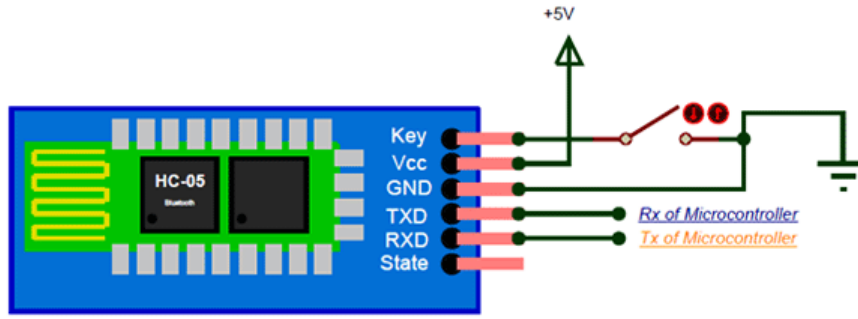

Fig 6 HC-05 Bluetooth Module pin out

The Hc-05 module operates at 5 V or 3.3 V power supply. The Key pin is used to bring Bluetooth module in AT commands mode or data mode. If key pin set to high, it operate in command mode, that change setting of HC-5 to send these commands to serial port is used. Otherwise itis in data mode to exchange of data between devices. The baud rate of HC-05 in command mode is 38400 bps and 9600 bps in data mode. TXD and RXD pins acts as an USART interface for communication. The data is transmitted serially out through TXD pin; it is connected to RXD pin of PIC controller. While the serially data received through RXD pin by the module from TXD pin of PIC controller. The state pin is connecting to LED to indicate Bluetooth is working properly.

III. CONSTRUCTION AND WORKING

The SW10 soil moisture sensor buried 4 inch to 6 inch deep at root zone of crops in field, available DHT11 humidity sensor is installed near the crops in farming field. The data from moisture, humidity and dry run sensors fed to the HC-05 Bluetooth module through PIC-16F777A controller. The outcome data from the PIC controller is connected to the LCD display unit, electric motor and fertilizer valve. The Bluetooth module wirelessly communicates with smartphone and controller shown in fig 7.

The working of the circuit is as follows, the soil moisture sensor measures the conductivity of the soil. The conductivity of wet soil is maximum than dry soil. This information received by the controller and sends to user's smartphone through Bluetooth, user can compare it and send the signal to controller to start the motor for crop watering. Thisappropriate message willdisplay onLCD, the output of the microcontroller is connected to therelay through base of transistor in driver circuit. If the output of controller is high then transistor is turn on, the relay coil gets energized and turn on motor as well as fertilizer valve. When the moisture of soil reaches the threshold value, the output of soil moisture sensor is low and motor will turn off.

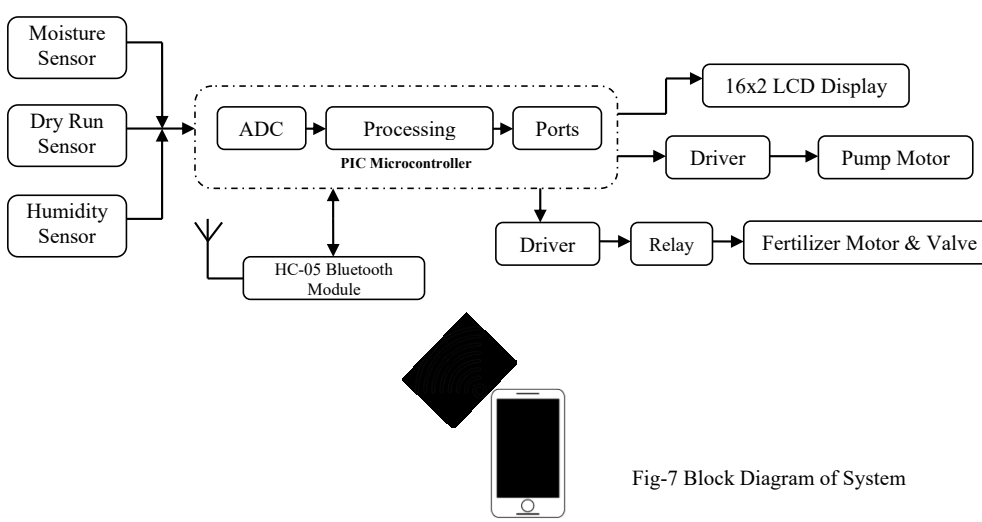

Fig-7 Block Diagram of System

1) Before watering:

In this process soil moisture sensor detect the volumetric water content in soil, thisSWC content

level is low means dry soil, due to electrical property maximum resistivity of dry soil and minimum current. Soil moisture sensor output gives to PIC controller; convert it into digital form in controller and this value goes below a fixed criteria (threshold) level then controller sent the information about dry soil on user smartphone through Bluetooth module. User can sent signal back PIC controller to start motor of drip irrigation for watering the crops.

2) After watering:

In this again soil moisture sensor measure the volumetric water content in soil, the SWC content level is high means wet soil, due to electrical property resistivity of dry soil minimum and maximum current. The output of the soil sensor gives to PIC controller; convert it into digital form in controller and this value of wet soil goes above a fixed criteria (threshold) level then controller sent the information about wet soil on user smartphone through Bluetooth module. Farmer (user) decide that to stop motor of drip irrigation by sending signal back to the PIC controller.

3) During watering:

In this water level sensor sense the water level in the well and sent the signal to controller. If water level goes below the sensorthen controller sent this signal to user and immediatelystop the motor. When water level goes above to the water level senor, controller sent this information of sufficient water level to user and user gives response back to controller to the motor

4) During Fertilizer:

The DHT11 humidity sensor is situated near farm, it detect the moisture and temperature in the air and send this signal to controller, which is also send to user smartphone. The farmer takes the decision according to humidity sensor information and sends the command to the controller to open valve of fertilizer pipe and start fertilizer motor.

5) No water in Well:

If there is no water in well, no load on electric motor which is at run high speed and draws the heavy current. Due to increased friction between rotor and stator, rise the internal temperature at stator then motor operate dry running. This rapidly rising temperature
is sensed by the FCTE45 heat sensor device and sent to the PIC controller, which is stop the motor immediately.

System had to carry out the semi-automatic irrigation that supplies water and fertilizers to roots of crop as per the environmental conditions. In this system automation is performed by the PIC-16F777A (Programmable Interface Controller) microcontroller.

RESULTS AND CONCLUSION

The crop growth and yield directly dependent on water amount in its body, critical growth of different crops at various stages for water supply to crops. According to types crop watering schedule can change, this system provides the correct frequency and duration of watering the crops.The scheduling irrigation system provides the correct frequency and duration of watering the crops to minimize crop water stress, labor charges and maximize yields. Regarding the changes in weather conditions, system algorithm can handle the situation. If no water available in well motor run in dry condition then system will automatically turn off motor and avoid further loss.

The water and fertilizer are two important factors essential for growth of crops. It becomes easy to give sufficient dose of chemical fertilizer as Urea, potash to crop along with water through drip irrigation, resulting improves the quality. By checking the weather condition pesticides also supplied to the crop with this fertilizer to avoid the disease.This system provide the facility of motor protection against the motor damage when no sufficient water in well.

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CLOUD COMPUTING - DATA SECURITY CHALLENGES& SOLUTIONS

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ABSTRACT: *Cloud computing is one of the fastest raising technologies in computing. There are many benefits as well few security challenges in cloud computing. This paper explores the different data security challenges in cloud computing proposes methods to overcome the security challenges. In any business or Cloud Computing data are exceptionally important, data leaking or corruption can explode the confidence of the people and can lead to the collapse of that business. The Cloud is very effective way to manage the data of mobile devices as the data storage is one of the big control in small devices like cell phones. Still storing data on cloud that means gives total control of user data to cloud service provider. This may leads to embezzle of mobile data. To avoid this problem various data security techniques are introduced, so that user can give only responsibility to cloud service provider and not the accessibility of data. This paper presents overview of different challenges for the security of data on cloud. Currently cloud computing is used directly or indirectly in many businesses and if any data split has happened in cloud computing, that will affect the cloud computing as well as the company's business. That is why cloud computing companies to give more emphasis on data security.*

Keywords: *Data Security, Cloud Computing, Encryption, Confidentiality, Integrity, Availability and Access Control*

I. INTRODUCTION

By choosing the cloud services the users are able to store their local data in the remote data server [2]. The data stored in remote datacenter can be accessed through the cloud services provided by the cloud service providers. Cloud Computing security is the major concern to be used now a days. If security measures are not provided properly for data operations and transmissions then data is at high risk [3]. Cloud computing is shared pools of configurable computer system resources and higher-level services that can be rapidly provisioned with minimal management effort, often over the Internet. Cloud computing relies on sharing of resources to achieve coherence and economies of scale, similar to a public utility [5].

One of the new emerging technology is cloud computing which is providing solutions for services like accessing or working with the various cloud applications. We can access the cloud data from anywhere if our application is connected to the internet as cloud computing is providing a way to store and access the data.

II. DATA SECURITY CHALLENGES

As we are moving into internet based cloud model, it requires great significance on Data Security and Privacy. Data loss or Data leakage can have severe impact on business, brand and trust of an institution.

1. Security

The security and Privacy are the main challenges to the cloud computing. A number of security threats are associated with cloud data services not only traditional security threats, such as network eavesdropping, illegal invasion, and denial of service attacks, but also specific cloud computing threats, such as side channel attacks, virtualization vulnerabilities, and abuse of cloud services[6]. Hacking and various attacks to cloud infrastructure would affect multiple clients even if only one site is attacked [7].

2. Interoperability and Portability

Interoperability means the ability of computer systems or software to exchange and make use of information. Cloud Portability is the ability to move applications, software's and data from one to another cloud computing environment. Cloud computing services should have the capability to integrate smoothly with the on-premise it [7].

3. Cost

The cost required for software licenses, physical servers, contact of maintenance, hardware warranties, material, spare parts etc. The cost of transferring the data is a problem for small scale projects. Companies can save some money on system maintenance, management, and acquisitions but they also have to invest in additional bandwidth, and the absence of routine control in an infinitely scalable computing platform can increase costs [8].

4. Service Provider Reliability

The capacity of a technical service provider is important like price. The service providers are available to provide data
from cloud when we need. The service provider's maintainability is related to the reliability of service provider. Make sure you comprehend the techniques via which a provider observes its services and defends dependability claims [8].

5. Vicinity

In cloud computing, the data is distributed over the number of regions and to find the location of data is not easy. When the data is moved to different geographic locations the laws governing on that data can also change. So there is an issue of compliance and data privacy laws in cloud computing. Customers should know their data location and it is to be intimated by the service provider [4].

6. Integrity

The system should maintain security such that data can be only modified by the permitted person. In cloud based environment, data integrity must be maintained correctly to avoid the data disorientation. Most of the web services face lot of problems with the transaction management frequently as it uses HTTP services, HTTP service does not support transaction or guarantee delivery [4]. Every transactions in cloud computing should follow ACID Properties to preserve data integrity.

7. Migration issues

If migrating data from system to cloud not properly gives risks. There is a need for developing a migration policy for solve this problem.. Companies often find it difficult to pick the appropriate service model for their businesses and prefer to associate with providers who allow the creation of customized computing environment [9].

8. Continuous monitoring and supervision

There is a problem of interruption of service, electrical failure when data is stored on cloud. To avoid this problem there is need of monitoring and supervision. It is important to monitor the cloud service continuously as well as to supervise its performance, business dependency, and robustness [9].

9. Separation

Data can be obstruct by introducing client data or software hence there is a need to place data separately. Vulnerabilities with data segregation can be detected or found out using the tests such as SQL injection, Data validation and insecure storage [4].

III. SOLUTIONS TO DATA SECURITY CHALLENGES

The process of translating plain text data into cipher text is known as encryption. The best solution to secure the information is Encryption. And the process to convert cipher text to plaintext is known as decryption. It is better practice to store the cipher text form data in cloud .The permission is given to the each of the group member by data owner so that they can easily access the data easily. Data access control is provided by heterogeneous data centric security.

Data encryption, decryption, authentication, data recovery, user data protection techniques are used to improve the data security in cloud. To ensure privacy and data security data protection is used.Because of applying encryption on data it will make the data totally unusable and normal encryption can complicate availability. A data driven framework can be designed for secure data processing and sharing between cloud users, Network based intruder prevention system is used to detect threats in real-time as well as To compute large files with different sizes and to address remote data security RSA based storage security method can be used[4].

Before uploading data into the cloud the users are suggested to verify whether the data is stored on backup drives and the keywords in files remain unchanged and then calculate the hash of the file before uploading to cloud servers will ensure that the data is not altered then this hash calculation can be used for data integrity but it is very difficult to maintain it [4]. RSA based data integrity check can be provided by combining identity based cryptography and RSA Signature [4].In cloud computing distributed access control architecture can be used for access management. Credential based policies are used to identify unauthorized users.

IV. CONCLUSIONS AND FUTURE WORK

Although cloud computing is the new appearing technology that presents a good number of benefits to the users, it faces lot of security challenges. There is a scope to propose the guidelines to overcome

the future challenges like physical security, transparency, data ownership, hypervisor viruses and malicious insiders in Cloud security. In future tangible standards for cloud computing security can be developed. To provide a secure data access in cloud, new encryption techniques can be used for storing and retrieving data from cloud. Proper key management techniques can be used to distribute the key to the cloud users such that only authorized persons can access the data.

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KNOWLEDGE REPRESENTATION FOR LEGAL INFORMATION NETWORK USING GRAPH DATABASE

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ABSTRACT: *Law is profession of rules and procedures, in order to understand a legal case, one has to gothrough a lengthy and tedious process. Also it is time as well as effort consuming task to search manually the required similar judgments for the citation. The goal of this project is to build the framework capable of detecting and modelling knowledge directly from the judgment's text, providing the basic metadata to the logic and reasoning layers using unsupervised classification and further clustering them as per user search criterions. This legal system shows how to model judgments starting from the text and capturing the structural parts. This project does not use any sql database or cloud for storing judgments. There is graph based access to data which provides a better GUI for interaction with users. For finding citations, pattern matching is done using regular expressions our proposed system creates a network citation graph for a judgment, in which a judgment is represented as a node and provides relationship between judgments according to similarities.*

Keywords: *Network Citation Graph, Incitation, Graph Database.*

I. INTRODUCTION

In a common law system, which is currently prevailing in countries like India, England, and USA, decisions made by judges are important sources of application and interpretation of law. The increasing availability of legal judgments in digital form creates opportunities and challenges for both the legal community and for information technology researchers. While digitized documents facilitate easy access to a large number of documents, finding all documents that are relevant to the task at hand and comprehending a vast number of them are non-trivial tasks. Also, there is a rising need for effective information retrieval tools to assist in organizing, processing, and retrieving the legal information and presenting them in a suitable user-friendly format. This system provides a network citation graph of a judgment. Citation is a reference to a published or unpublished source. Here in this context a network citation graph refers to a directed graph in which each vertex represents a document (judgment) and an edge represents a citation from current document to another. For example judges refer to earlier judgments to support their decisions.

Knowledge representation refers to getting the useful data from the text and representing it in a form which can be used by the computer system for solving complex problems. So in this project by applying some logic we extract some of the particular type of information as for a judgment whole judgment story is not important, the facts which make it important are petitioner, respondent, date of judgment, judge, acts violated, type of punishment, citation of 6 different judgment. Getting information regarding this attribute is metadata of that judgment which makes it knowledgeable. So this project tries to get all the information which makes it more informant as itself.

To store this entire information, graph database is used. The graph database not only saves data in text format, but it also creates nodes having different types of labels, different types of relationship. Then as the parameters occur in the judgment it creates nodes as per the satisfying condition and also create relationships between two nodes according to similarities.

The created graph database is named as legal information network which contains all judgments and its corresponding features with relationship which look like the network of nodes connected to different types of nodes with special labels. In this network there will be some node which contains more no of relationships as compared to its type of nodes. Then it will create some additional information after graph creation such as which judgment is frequently cited?, the count of judgments given per day/month/year,

count of type of Courts, which act is frequently violated in particular type of judgments, etc. Likewise all the information can be collected after the creation of the graph database, which cannot be derived from the plain text.

Second section explains existing system for the judgment retrieval which has been in use for the past years. Third section describes the proposed system architecture of the project, it describes its working all modes and labelling of feature vectors to create the graph database. Fourth section is the conclusion of the project.

II. EXISTING SYSTEM

The existing system is that the lawyers have to go through tedious manual work for finding out the judgments relevant to particular case. This traditional process has many drawbacks. It is very tedious and time consuming work. There are also some software's that provide judgment searches. But these software's provide text based search throughout all judgments without considering the meaning of that word, which gives large number of irrelevant documents as a result and it is hard to find out what actually was required as output of the search. Speed and efficiency these are the two factors lacking in the existing system.

III. PROPOSED SYSTEM

This systems involves web based UI as frontend to interact with users and the graph database as backend for storage and retrieval. Further this system act as an artificial intelligent system which will automatically grow its graph database as the number of searches increases.

A] System Architecture

This system contains two phases namely training phase and predicting phase .First we train the system on some judgments and then test on new judgments.

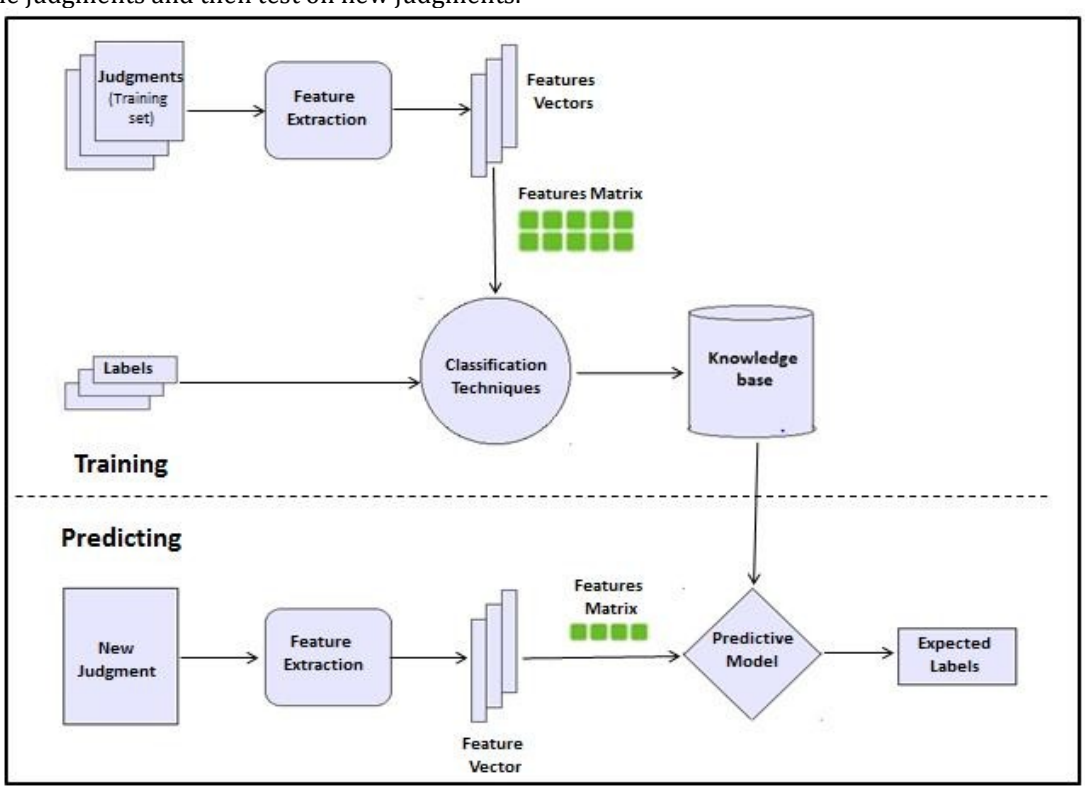

Fig 1: block diagram of proposed system

In training phase, html pages of judgments are given as input to the system where we extract features from judgment. Some of the features are name of petitioner, name of respondent, date of judgment, judge, act, type of court, etc. The extracted features are considered as the feature vectors. This system extracts feature vectors of all judgments so the collection of feature vectors is the feature matrix. This feature matrix is fed as input to the classification algorithms where the different types of classification techniques are used to map feature vector corresponding to its label. Some of the techniques used are SVM (Support Vector Machine), Naïve Bayes algorithm, etc.

The SVM (Support Vector Machine) classifier is used for N-way classification. SVM are supervised learning models with associated learning algorithms that analyze data used for classification and regression analysis. Given a set of labels as training examples, each marked as belonging to one or the other of two categories, an SVM training algorithm builds a model that assigns new examples to one category or the other, making it a non-probabilistic binary linear classifier. An SVM model is a representation of the examples as points in space, mapped so that the examples of the separate categories are divided by a clear gap that is as wide as possible. New examples are then mapped into that same space and predicted to belong to a category based on which side of the gap they fall. The classified data is stored into the knowledge base.

Knowledge base is a technology used to store complex structured or unstructured data used by computer systems. Here in this context legal information base is stored as graph database. There will be various labels such as Act, name of judge, date of judgment etc. These labels along with extracted features are given as input to the classification techniques. If the label parameters are present in the feature vector then that particular label is added to the judgment as extended label. Depending upon the number of extended labels added to the judgment, relationships among the judgment and the labels are created to be stored in graph database. For example, suppose Hindu marriage act is one label. If the words such as Hindu act, Marriage Act, Hindu Marriage act, etc. are present in the feature vector of particular judgment then label is added to that judgment.

In graph database, there are nodes present of various labels such as different types of acts, judge, judgment, date, type of court, etc. According to the extended labels added to the judgment if the particular labels condition is satisfied then an edge is created between these two nodes marked with that particular relationship example: In-citation, Out-citation, date of judgment, etc. In this way as no of labels increases for particular judgment then graph expands automatically and this graph is nothing but legal information base.

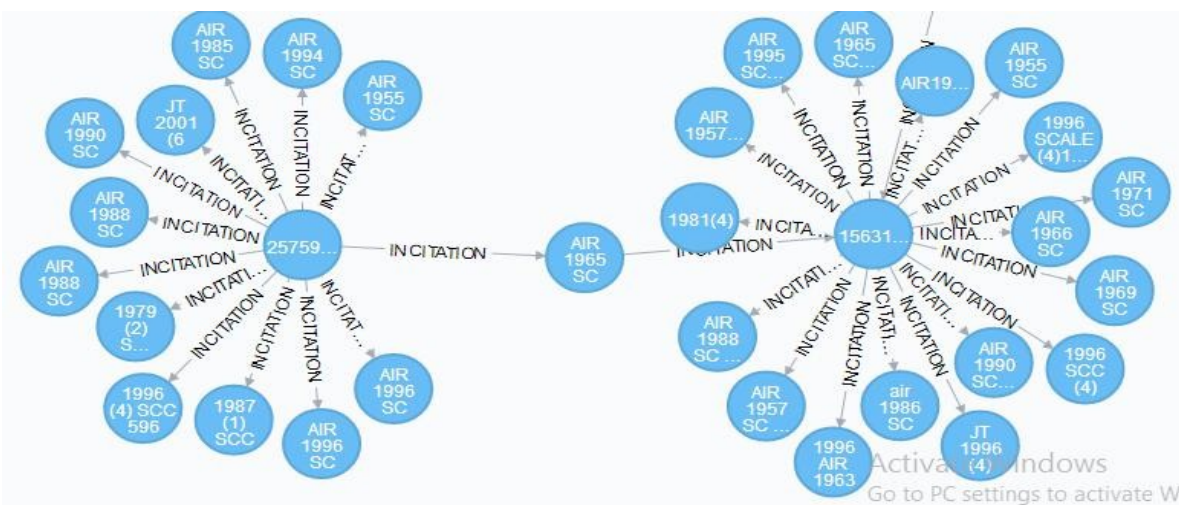

Fig 2: graph database of in-citation relationship

As particularly above graph database contains judgment labeled node and having In-citation relationship among the two judgments. As means if In-citation label is added to the judgment corresponding of particular citation of judgment then the citrated judgment node is created and relationship between the judgments is stored as In-citation relationship. Like this all relationship created corresponding to the added expected labels of the judgment

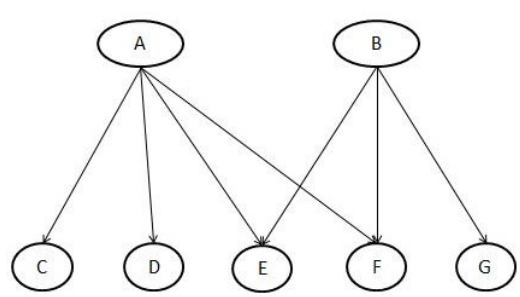

Fig 3: An illustration of case-citations of two Judgments.

Citation network graph explains two main properties of relationships namely ***In-citation*** and ***Out-citation.*** Typically 'case citations' contribute towards the argument of thejudgments by leveraging the legalconcepts of the cited judgments. Hence, all the cited judgments go through strict scrutiny of legal experts, during argument between two opposing lawyers. For a given judgment there are two types of 'case citations', which are explained below:

Out-citation of a Judgment: For a given judgment 'J', we define out-citations as those 'case citations'which are mentioned in judgment 'J' and are referring other judgments. In Figure 1, judgment 'A' has four out-citations namely judgments 'C','D','E' and 'F' while judgment 'B' has three out-citations namely 'E','F' and 'G'.

In-citation of a Judgment: For a given judgment 'J', we define in-citations as those 'case citations' whichare referring to judgment 'J'. In Figure 1, judgment 'A' is in-citation for judgment 'C','D', while judgments 'B' is in-citation for judgment 'G'. Judgments 'A' and 'B' both are in-citations for judgments 'E' and 'F'.

After the training phase of system is completed then in predicting phase we provide new judgment as an input to the system from this new judgment features are extracted .These feature vectors along with the predictive model analyze how the labels are given to the feature vector in the existing knowledge base. Then predicting it similarly it tries to label the feature vector as like it is present in knowledge base. Sometimes it also creates its new labels by analyzing knowledge base for newly encountered features of judgment. Finally it gives a judgment with added expected labels and also store this expected corresponding to its node in the existing knowledge base for future reference. As the number of new judgments given for the predicting phase increases, the legal information network expands dynamically.

IV. CONCLUSION

One of the features of this system is the notion of understanding the terms which are relevant to the legal concepts. Thus, a knowledge engineering approach has been attempted. In our approach, we have created a ontological framework; through this we have mapped the keywords of Single domain with different relations. This framework could be extended to any domain depending upon the way we extract the features and using complex visualization concepts of Network Citation Graph. Web crawler is used for efficient and dynamic judgment search. Thus we have presented a smart legal system which will reduce efforts of lawyers and common man.

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BASICS OF ARTIFICIAL NEURAL NETWORK

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ABSTRACT: *One of the important branch of artificial intelligence is Artificial Neural Network (ANN).This paper proposes a combination of traditional airborne computer systems with neural network processing modules. In recent years most of the pattern recognition and machine learning highlighting on Artificial Neural Networks (including recurrent ones). The problems like anticipating and analyzing flows in which traditional methods and statics aren't able to solve artificial neural networks have been approved. In this article, by using feed forward network with transmission function in input and output layers. An ANN is used for a specific application, such as pattern recognition or data classification, through a learning process. Learning process in biological systems includes adjustments to the synaptic connections that exist between the neurons. This paper gives overview of Artificial Neural Network, working & training of ANN.*

Keywords: *Artificial Neural Network, learning, biological systems, Feed forward network.*

I. INTRODUCTION

The ANN is introduced on the basis of biological neural network which plays important role in human body. Human brain handles all the work done in human body. Neural Network is nothing but web of interconnected neurons which are millions and millions in number. In human body all the parallel processing is done with the help of the interconnected neurons and human body is the best example for parallel processing.

The basic processing element of a neural network is a neuron. Basically, a biological neuron receives inputs from other sources, combines them in some way, performs a generally nonlinear operation on the result, and then outputs the final result[3]. Below Figure shows the relationship of these four parts. A neuron process information from one neuron to another neuron with the help of some electrical and chemical change. It is formed by a cell body or soma and two types of out reaching tree like branches: the axon and the dendrites. Each cell body has a nucleus that contains information about hereditary traits and plasma that holds the molecular equipments or producing material needed by the neurons. A neuron receive signals from other neuron through dendrites as the whole process of receiving and sending signals is done in particular manner. The soma then processes these incoming signals over time and the soma then turns that processed value into an output which is sent out to other neurons through the axon and the synapses [3].

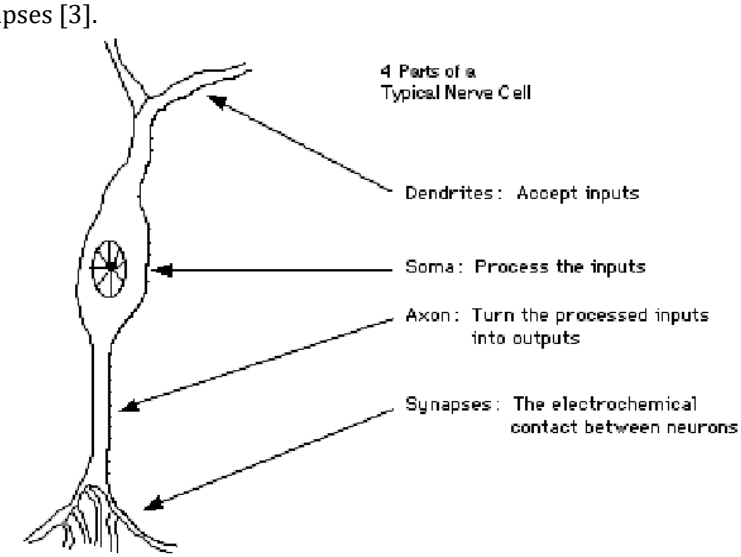


Figure: A Simple Neuron [3] 1

A. What is Artificial Neural Network?

These basically consist of inputs (like synapses), which are multiplied by weights (strength of the respective signals) and then computed by a mathematical function which determines the activation of the neuron [1].

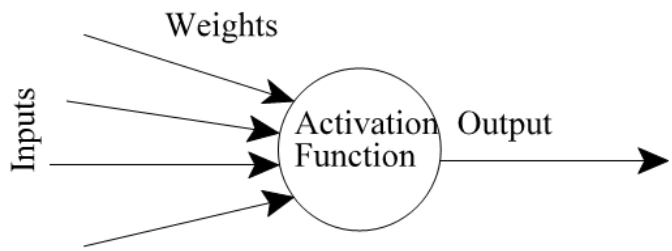


Figure: An Artificial neuron [1] 1

By adjusting the weights of an artificial neuron we can obtain the output we want for specific inputs[1].As like the biological Neuron ANN also have neurons which are artificial and they also receive inputs from the other elements or other artificial neurons and then after the inputs are weighted and added, the result is then transformed by a transfer function into the output.

B. Why to use Artificial Neural Network?

The excellent characteristics to brain of human being which are not present in modern computers which are:-

- Parallel information processing
- Distributed representation and computation
- Adaptability
- Learning Ability
- Generalization Ability
- Inherent Contextual Information Processing
- Fault Tolerance[2]

It is supposed that devices which are based on biological neural networks will acquire at least some of these desirable characteristics of human being. Digital computers can perform numerical computations and symbol manipulation outstandingly. But human beings can easily solve the problems like identifying the person in the crowd who is having spots on his face in such high speed that any of world's fastest computer can do.

Taking inspirations from biological neural networks ,ANN's are the one which are massively parallel computing systems which consists of large number of simple processors which are interconnected to each other. ANN models can use some of "institutional" principles that humans can use.

II. Network architectures

Representation of ANN is done by using weighted directed graph in which artificial neurons represents nodes of the graph and directed edges (with weights) shows connection between neuron output and neuron input. Based on how the nodes are connected to each other they can be grouped into two categories:

* Feed-forward networks
* Recurrent networks

In feed-forward networks, which are known as multi-layer perceptron and are unidirectional connection between them with organization of multiple layers.

As the connectivity changes the networks have different behaviors, feed-forward networks produce only one set of values rather than sequence of values from given input and hence are static. They have no memory as the response to an input is independent of the previous network state. Recurrent, or feedback, networks, on the other hand, are dynamic systems, when a new input pattern is presented, the neuron outputs are computed. Because of the feedback paths, the inputs to each neuron are then modified, which leads the network to enter a new state [2].Appropriate learning algorithms are used in different network architectures.

III. Learning

Learning is the process of acquiring new, or modifying existing knowledge, behaviours, skills, values

or preferences. The ability to learn is a fundamental aid of intelligence. In ANN Learning can be implemented by updating the network architecture and connection weights in such a way that network can perform particular task efficiently.

The network usually must gets the connection weights from available training patterns. Performance is improved over time by iteratively updating the weights in the network, ANNs' ability to automatically learn from examples makes them attractive and exciting [2]. Instead of following a set of rules specified by human experts, ANNs appear to learn underlying rules (like input-output relationships) from the given collection of representative examples. This is one of the major advantages of neural networks over traditional expert systems [2]. To understand or design a learning process, we must first have a model of the environment in which a neural network operates, that is we must know what information is available to the network.

Learning can be of two types:

Supervised Learning:

In Supervised Learning machines learn by some initial provided labelled data inputs sets. Machine can learn by recognizing and matching the patterns with initial provided data.

Unsupervised Learning:

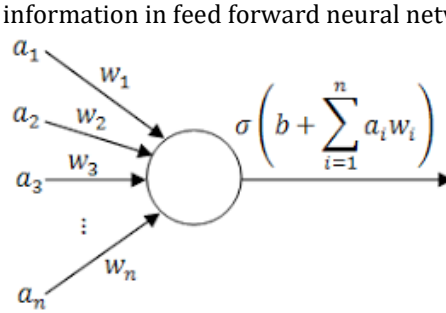
In Unsupervised Learning no initial labelled data is provided to the machine. Machine can learn by determining the hidden pattern or grouping in data from unlabeled data.

IV. Feed-forward Neural Networks

Feed-forward Neural Networks is a type of Neural Network Architecture where the connections are "fed forward" that is do not form cycles. These are the simplest type of the artificial neural network. Feed forward can travels only in forward direction and hence called as feed forward, first through the input nodes, then through the hidden nodes (if present), and finally through the output nodes[2].Often the units in the neural network is also called as nodes. Data enters at the inputs and passes through the network, layer by layer until it arrives at the output.

In Feed forward neural networks data to be learned is neither sequential nor time-dependent. It is used for supervised learning. Feed forward neural networks compute a function on a fixed size input.

However, feedback neural networks learn sequential data, computing on variable length input such that for training pairs for all. Flow of information in feed forward neural networks as shown in below figure:



Single Layer 1

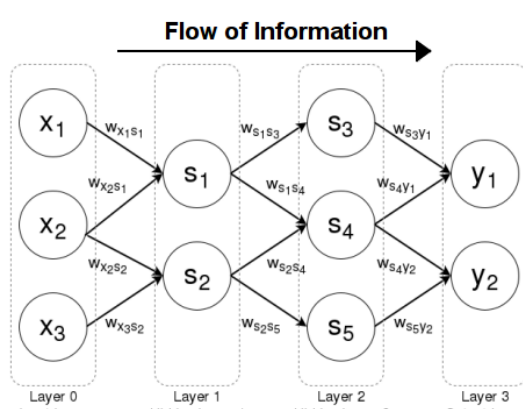


Figure: Multi-Layer Network 1

V. Recurrent Neural Network / Feedback Neural Network

It is a class of artificial neural network where connections between nodes form a directed graph along a sequence. A common method of neural network in which the initial system output is compared to the desired output and the system is adjusted until the difference between the two is minimizes hence called recurrent or backpropagation.RNN are dynamic in nature. RNN's are having internal states which will nothing but act as memory to store and process the sequence of inputs, in such a way they can be used for speech recognition, pattern recognition, hand-writing recognition etc. The term "recurrent neural network" is used indiscriminately to refer to two broad classes of networks with a similar general structure, where one is finite impulse and the other is infinite impulse [4]. Both classes of networks exhibit terrestrial dynamic behavior. A finite impulse recurrent network is a directed acyclic graph that can be unrolled and replaced with a strictly feed forward neural network, while an infinite impulse recurrent network is a directed cyclic graph that cannot be unrolled [4].Finite impulse and infinite impulse recurrent networks can have additional stored state, and the storage can be under direct control by the neural network. The storage can also be replaced by another network or graph, if that incorporates time delays or has feedback loops, such controlled states are referred to as gated state or gated memory, and are part of long short-term memory networks (LSTMs) and gated recurrent units [4].

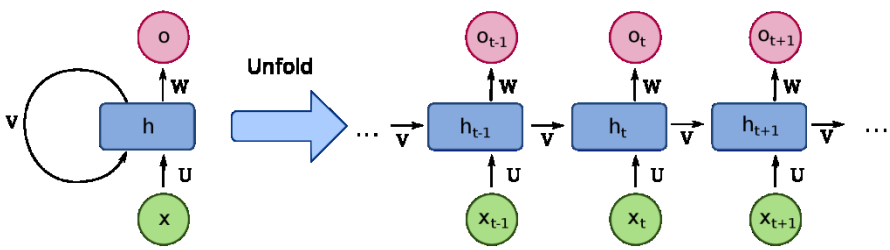


Fig. Recurrent Neural Network

VI. CONCLUSION AND FUTURE WORKS

By studying ANN we had concluded that it is the technique of mapping the human brain capabilities to provide intelligent computers to some extent. As technology is developing day by day the need of Artificial Intelligence is increasing because of only parallel processing. In today's world Parallel Processing is needed because with the help of parallel processing only we can save more and more time and money in any work related to computers and robots. In future work we can only say that we have to develop much more algorithms. Artificial Neural Network concepts combined with the Computational Automata and Fuzzy Logic we will definitely solve some limitations of this excellent technology.

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4. Website- https://en.wikipedia.org/wiki/Recurrent_neural_network

CYTOTOXICITY OF FUNGICIDE KREESOXIM-METHYL 44.3% ON ONION *(ALLIUM CEPA* L.) ROOT TIP CELL

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ABSTRACT: *Cytotoxicity of Fungicide KRESOXIM – Methyl 44.3% were calculated in the root meristem cells of Allium cepa. In the Allium root growth test, the effective concentration value was determined as approximately 5%. Cytological experiment were carried using KRESOXIM-methyl concentrations of 1%, 2.5%, 5%, at 24 hours with a control for each combination. Mitotic index value was progressively increased with increase of fungicide concentration. The onion root meristem treated with KRESOXIM-Methyl 44.3%of 1%, 2.5%, and 5% induced mitotic abnormalities like Anaphase bridge, binucleate cell, heteromorphic chromosomes, disturbed chromosomes, polyploidy metaphase, micronucleate cell, clumping at anaphase, fragmentation at metaphase while the control showed normal metaphase, anaphase, telophase according to total cells with mitotic index calculated as 11.77%, 14.65%,15.10% and 18.28%.The present investigation anaphase in mitotic division seen in more in number, hence the these fungicide (KRESOXIM-Methyl 44.3%) affect mitotic division to fix the anaphase stage.*

Keywords: *Allium cepa, fungicide, mitotic index, cytotoxic effect.*

INTRODUCTION :

Generally in agriculture farmers use pesticides to increase their yield by reducing crop losses by pests, some chemical compounds used pesticides are mutagens Fungicides are metabolic inhibitors and their modes of action can be classified into different group inhibitors of nucleic acid metabolism, protein synthesis and sterol synthesis. Fungicides are most commonly used against diseases of agricultural crops in many countries of the world.

Fungicides may also influence to change plant genetic system due to their mutagenicity and carcinogenicity. Dithane M-45 had the ability to cause production of large number of mitotic abnormalities (Maity S.K, 2014). Mutation breeding is a fundamental and highly successful tool in the global efforts of agriculture to feed an ever increasing and nutritionally damaging human population (Raina et al, 2016).The fermented Garri extracts have mitodepressive effect on cell division in Allium cepa. (Daniel J. Olorunfemi & Emmanuel O. Ehwre, 2010).

The *Allium cepa* assay is an efficient test for chemical screening and in situ monitoring for genotoxicity of environmental contaminant .this test has been used widely to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems of *Allium cepa*. (Donatella et al, 2007).The root tips several of plant species have been used for the study of induced chromosomal aberrations (CAS) and presence of micronuclei (MNi), (Hilada Nefic et.al,2013)

Onion (*Allium cepa*) is one of the major bulb crop of the world, which is grown in India. Onion has been considered as rich source of carbohydrates and minerals like phosphorus, calcium. It also contains protein and vitamin C. It is used for its flavor and pungency in daily food. Fungicides may also influence to change plant genetic system due to their mutagenicity and carcinogenicity. KRESOXIM-Methyl 44.3% fungicide it is kind of non-imbided and broad bactericide and high efficiency low toxicity, it is widely used in prevential land cure of plant mycosis for vegetables, fruit,tree, flowers, tobacco, tea,tree and other economical plant.

MATERIAL AND METHODS:

The plant used as test material was *Allium cepa L*. The root meristems of *Allium cepa* consists of diploid (2n = 16) set of chromosome. Clean and healthy bulbs of onion were chosen for each treatment group. Mature dry bulbs of onion were used as a experimental material to study the effect of KRESOXIM-Methyl 44.3% on onion root meristem cell. Healthy and uniformed sized bulbs of onion were placed at the mouth of 50 ml beaker filled with distilled water and kept in room temperature. As soon as root of the sprouted bulbs attained the length of above 0.5cm the bulbs were transparent to beaker containing different concentration of KRESOXIM-Methyl 44.3% (0, 1%, 2.5%, 5%). They were treated the test solutions for 24

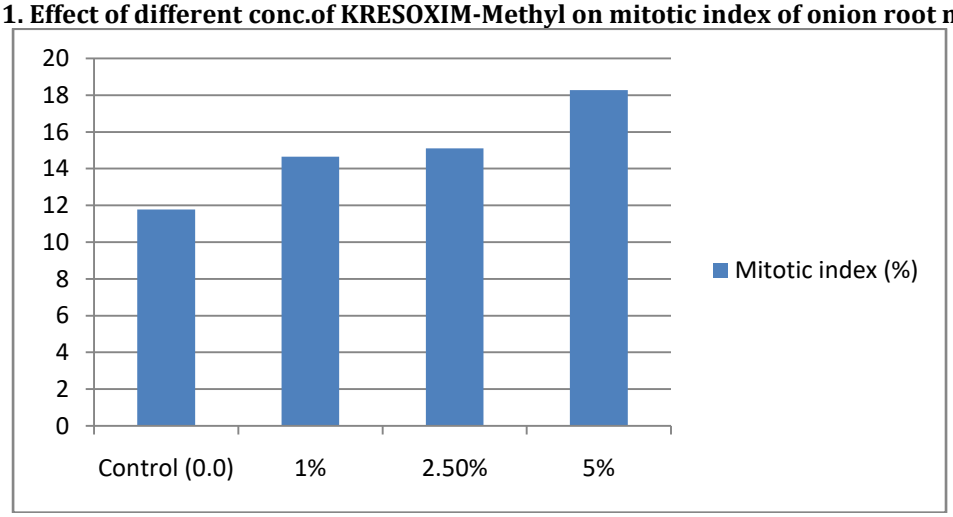
hours at room temp.

After the treatment in the fungicide onion bulb were washed under distilled water and immediately transfer to containing distilled water. They were allowed to grow for 2-3 days. Roots from treated onion bulbs were excised and root tip are cut and add to distilled water. the root tip are directly used or preserved in 1 part of acetic acid 3 parts of alcohol (acetic acid: alcohol 3:1) After 10 minutes preserved root tip are transferred to 75% alcohol, then after 10 minute wash root tip with distilled water. The root tips are kept in 1N HCL and warm for 1-2 min. The treatment HCL (hydrolysis) dissolves the middle lamella and cells get separated. It also allows better penetration to stain. After hydrolysis (root tip becomes translucent) stain with 1% aceto-carmine for 10-15 min. The Aceto-carmine reagent used to study cell division with special references to chromosomal and mitotic abnormalities.

RESULT AND DISCUSSION:

Conc. of KRESOXIM Methyl	Total no. of cells observed	Total no. of dividing cells	Mitotic index (%)
Control (0.0)	106	9	11.77
1%	191	28	14.65
2.5%	245	37	15.10
5%	268	49	18.28

Fig 1. Effect of different conc.of KRESOXIM-Methyl on mitotic index of onion root meristem



Mitotic Index

Data on the effect of concentrations control, 1%, 2.5% and 5% of treated 24 hours at room temperature .On the mitotic index of onion root meristem is shown in table.1 from the table in controls 11.77%.Some onion root meristem treated with KRESOXIM-Methyl 44.3% for 24 hours room temperature shows increase the mitotic index in 1% treated meristem KRESOXIM-Methyl the mitotic index was 14.65, 2.5% was 15.10 and also 18.28 in 5% treated. Mitotic index value was progressely increased with increase of fungicide concentrations.

Some mitotic abnormalities were observed the KRESOXIM-Methyl 44.3% treatment in control the onion root meristem observed normal phases, such as prophase, metaphase and telophase. Some mitotic abnormalities were observed the 2.5% KRESOXIM–Methyl 44.3% showed the mitotic abnormalities like disturbed chromosome at metaphase, anaphase bridge, heteromorphic chromosome at metaphase, abnormal anaphase, binucleate cell.

In the present study MSMA induced wide-range clastogenic effects like chromosomal and chromatic breaks,'subchromatid' (interchromatid) connections and heteromorphic chromosomes at metaphase; chromatin bridges and chrosomes fragmentation at metaphase. Micronuclei observed with the three pesticides are resultants of clastogenic events (Sparrow and Singleton 1953).Chromosome stickness and clumping in metaphase and anaphase and anaphase bridge were also observed,and might have been formed

due to the sticknieess at anaphase or due to the formation of dicentric chromosomes as a result of breakage and reunion.similar anaphase bridge were also noticed in the usage of insecticide isopropyl-N-phenyl carbamate (IPC), urethanes (Ennis 1948,Bruhin and Warner 1954).

The abnormalities observed were despiralization, spindle disturbances, Chromosome stickiness, lagging, bridges, *etc (*Shehab and Adams (1983).The abnormalities observed were clumped metaphase, scattered metaphase, polyploidy, diagonal anaphase, asynchronous movement of chromosomes, ball metaphase, *etc* Minija *et al.* (1999)

CONCLUSION:

Effect of the fungicide KRESOXIM-Methyl 44.3% on mitotic index on onion (Allium cepa L) root meristem was investigated under laboratory conditions. Use of fungicide KRESOXIM-Methyl 44.3% is widely used in prevential and are of cure of plant mucosis for vegetables, fruit, tree , flower and other economical plant.It is non- imbided and broad bacteriocide and high efficiency, low toxicity. It is applied to industry and antiseptic for dope,wiring,paper and cloth etc. As the mitotic index is a direct measure of rate of cell division in any organism and it proves to be an valuable parameter to assess the cytotoxic effect of any chemical agent ,the mitotic was as curtained for control and KRESOXIM-Methyl 44.3% onion root meristems.

The control, root meristems of onion, exhibited a mitotic index of 11.77%. same onion root meristem treated with KRESOXIM-Methyl 44.3% for 24 hour at room temperature showed decrease of mitotic index. In 1% KRESOXIM-Methyl 44.3% treated meristem the decrease in mitotic index was 14.65% and in 2.5%was 15.10% and also it was 18.25% in 5% KRESOXIM-Methyl 44.3% treated once. Mitotic index value has progressively increased with the increase of fungicide conc. Thus KRESOXIM-Methyl 44.3% is very effective in the mitotic indices of onion root meristem. The effect is dose dependent. Our studies on effect of KRESOXIM-Methyl 44.3% on mitotic index in onion root meristems warrant further investigations on this agrochemical on a more intensive and extensive basis, especially on crop plants, since it can affect the growth rate and interferes with cell division. The present investigation anaphase in mitotic division seen in more in number, hence the these fungicide (KRESOXIM-Methyl 44.3%) affect mitotic division to fix the anaphase stage.

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DIVERSITY OF FUNGI ASSOCIATED WITH LEAF LITTER OF MANGIFERA INDICA LINN. FROM GANGOBA SACRED GROVE, HASANE, RADHANAGARI (DIST. KOLHAPUR)

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ABSTRACT: *Sacred groves are considered as sacred natural sites which represent a diverse spectrum of ecosystem. These groves attain significance due to providing ecological services to local landscapes. All components of vegetation are supported to provide voluminous leaf litter which undergoes the process of decomposition by fungal communities. Presently attempt has been made to explore and investigate leaf litter fungi from one of the important angiospermic plant Mangifera indica Linn. from Gangoba sacred grove, Hasane from Radhanagri Tehsil (Dist. Kolhapur). Fungi decomposing leaf litter of forest plants play vital role in recycling of nutrients contained in the leaf litter of sacred grove forest. Ten species of fungal communities are isolated by culture method and identified with the help of standard literature. The leaf litter of Mangifera indica Linn. in two phases resulted, 3 species belonging to Ascomycotina, 2 species from zygomycotina and 5 species from Deuteromycotina responsible for decomposition of leaf litter.*

Keywords: *Leaf litter Fungi; diversity; sacred grove; Mangifera indica.*

INTRODUCTION:

Sacred groves vegetation is always rich in plant diversity which serve diverse spectrum of ecosystem. The forest floor of such sacred grove possesses rich leaf litter which undergoes a process of decomposition by variety of organism, among which fungi has vital role in degrading complex organic material of plant litter to similar products including nutrients. These are recycled in soil for growth of forest plants.

Thirty seven sacred groves were recorded from Radhanagari, Bhudargad, Ajara, Malkapur, areas of Kolhapur district (Anonymous, 1983-86). Gangoba sacred grove selected for exploration and investigation of fungi associated with *Mangifera indica* Linn. leaf litter from Radhanagari Tehsil. The presiding deity of the temple in the sacred grove is Gangoba. The area of the Gangoba sacred grove is near about 8 ha and is adjacent with a large tract of reserved forest of the sanctuary .This sacred grove is also located on the immediate eastern slopes from the crest of the Sahyadri hills and is surrounded by a large tract of non-forest area on its east. Geographical coordinates of Hasane are as 16˚ 20’ 45.75” N and 73˚ 51’ 19.55” E and Altitude -600 meters above msl, Temperature -28 ˚C, Humidity-90˚ %. Its weather is moist and salty during September to November. The leaf litter fungi are distributed in the tropical and subtropical region and also they are particularly dominant in forest area especially in rainy season. The sacred groves are reserved forest for deity, so vegetation of sacred groves is not affected by human disturbance. Therefore, climatic conditions of this sacred grove favor the growth of fungi on available leaf litter. The leaf litter fungi play efficient role in recycling nutrients. Decomposition of plant is a key step in nutrient recycling (Berg et al 2001).

The present investigation shows diversity of leaf litter fungi on decomposing leaves of *Mangifra indica* Linn. The investigation of leaf litter fungi studied in two different Phases, (Phase-I, II; Phase-I - initiating phase of decomposition, Phase-II- middle Phase of decomposition).

MATERIAL AND METHODS:

The leaf litter samples were collected randomly from forest floor of Gangoba Sacred Grove, Hasane in two phases during March and September months respectively. Samples were collected in sterilized polythene boxes and brought to the laboratory for further investigations. Host samples were identified using standard literature (Yadav and Sardesai 2002; Pascal 1987; Subramanian 1971). Identified samples are deposited in mycological herbarium of college by providing accession number.

Among the samples collected, leaf litter of *Mangifera indica* Linn. was selected for isolation of fungi , which was made by using culture method (Washing disks leaf technique of Sharma *et. al*, 2011).In washed disk technique 10 leaf disks were prepared in each phase . These disks were washed serially in three changes of sterilized distilled water and allowed to dry in air in chamber. Leaf disks were plated in two Petri plates containing Potato Dextrose Agar (PDA). The plates were incubated at 25 ± 1˚ C for 7 days. The fungal mycelium from each colony mounted on glass slides in lactophenol and stained with cotton blue in

lactophenol. Fungi are identified with the help of standard literature (Barnett 1987, Thom 1945, Tsuneo 1937; Ellis 1971; Subramanian 1971).Photomicrographs are taken by using Leica DM 2000 Fluroscence microscope equipped with digital camera.

RESULTS AND DISCUSSION

Taxonomic identification for leaf litter fungi associated with leaves of *Mangifera indica* Linn. showed the occurrence of 10 species belonging to Zygomycotina , Ascomycotina and Deuteromycotina. The details of taxonomic description are as follows.

Scedosporium prolificans (Hennebert and Desai) Gueho and de Hoog, J. Myco. Med. 118:3-9 (1991)
Synonym- ***S. inflatum*** Malloch and Slkin
Plate-1-A
Colonies grown on PDA , flat spreading , olive-gray to black, conidia borne in small groups on distinctive basally swollen, flask –shaped, occur singly or in clusters along the vegetative hyaphae ; conidia single celled hyaline to pale brown , ovoid to pyriform, smooth thin walled, Conidia 3-5×5-10 µm in diameter.

Scedosporium apiospermum (saccardo) Casteilani and Chalmers, J. of Cli. Micro. 43(10):4938 (2005)
Plate-1-B
Light yellow diffusible pigment on PDA after one week incubation , grayish –black reverse, numerous single-celled , pale-brown, broadly clavate to ovoid conidia , 4-9×6-10 µm in diam., rounded above with truncate bases conidia borne singly or in small groups on elongate, simple or branched conidiophores or laterally on hyaphae.

Pestalotiopsis hainanensis A.R. Liu, T. Xu and L.D. Guo. In Fun. Div. 24:23-36 (2007)
Plate-1-C
Colonies grown on PDA; white, cottony, acervuli black in color on mycelium with black spore mass; conidia fusiform, erect or slightly curved, 5- celled 19-25.2×5-6.3 µm, constricted at septa, central cells are brown in colour, thick walled, subcylindrical, second cell from the base pale brown, 3.2-4.4, µm and fourth cell pale brown to olivaceous, 2.9-5µm, terminal cell hyaline, small, trigonal, bearing 1 setula.

Mucor circinelloides van Tieghem , Domas. et al. 1980; Zych. et al. 1969
Plate-1-D
Colonies grown on PDA ; white at first turns greenish brown colour; mycelium coenocytic; sporangiophores hyaline, erect, mostly branched sympodially 1050×15µm ; Sporangia terminal, dark brown or black 22.5-37.5 µm in diam.; columellae echinulate or smooth, columellae hyaline globose or subglobose with collar 15-18.8×22.6-33 µm in diam.; Sporagiospores hyaline or pale yellow brown in mass, globose, subglobose, or ovate, 1-celled 7.5×5.6 -8.5×5.6 µm in diam.

Syncephalastrum **racemosum** (cohn) Schroet.
Plate-1-E
Colonies grown on PDA, light to dark gray, mycelium coenocytic, sporangiophores erect , with adventitious rhizoids, branching irregular , each branch apically dilated to form sporagiophores by septum , bear merosporangia directly over their entire surface , merosporangia rod shaped , many spored without a basal cell, wall evanescent at maturity, sporangiospores uniseriately arranged, globose or ovoid, sporangiospores 3-5 µm diam., ovate 54-6× 43-5 µm diam., zygospores globose , dark brown rough with broad shallow pointed projections.

Aspergillus violaceo-fuscus Gasperini , in Att. Soc. Toscana Sci. Nat. Pisa, Mem. 8,fasc.2,p.326(1887)
Plate-1-F
Colonies growing on PDA, purple brown with a faint violet shade; mycelium septate; conidiophores up to the 2 mm× 15-18 µm; conidial heads purplish brown, globose, not crowded 96.2to 119 µm in diam., vesicles globose varying up to 60.3-96.2 µm in diam., sterigmata in one series 5.5- 7.1×3.1 µm, conidia elliptical,4.7 to 5.5 ×3.1-3.9 µm, at first hyaline, becoming violaceous, somewhat roughened

Aspergillus japonicus Saito, in Bot.Mag. (Tokyo) 20:61, 5 figs. (1906)
Plate-1-G
Colonies growing on PDA; mycelium septate ; purple brown heads; conidiophores 100 to 1500 × 15 µm, thick walled, brown; conidial heads purple –brown,heads 75-90 µm in diam., vesicles globose, fertile over the whole surface with walls brown and marked by the bases of sterigmata; sterigmata in one series , 7-9×5 µm; conidia globose, echinulate 4-6.3 µm in diam. and elliptical 4-6.3×4.7 µm in diam.

Aspergillus **niger** van Tieghem, in Ann. Sci. Nat. Bot., S.5, t.8, p.240 (1867)
Plate-1-H
Colonies grown on PDA, with submerged mycelium, colorless, conidial heads blackish- brown, in typically

globose or radiate, up to 100 µm in diam.; conidiophores arising from substratum, uncolored, yellow to brown near to the vesicles only ,smooth, with thick wall , 1500 to 1700 µm in length and 20-22.5 µm in diam., vesicles globose or subglobose, thick walled, 20- 50 µm in diam., sterigmata in two series, colorless at times, more or less brown , even carbonaceous, primary sterigmata closely packed , covering the vesicle, varying greatly in size in the same colony, but usually 11.06-15.5×4.7-5.7 µm in diam., secondary sterigmata more uniform ranging usually from 9.4×4.7 -10×5 µm in diam., both series more or less brown to black, Conodia globose, first smooth with diffused brown or fuscous color then rough or spinulose ,bars or loops between outer primary wall and inner, 2.3-6.3 µm in diam., elliptical 4.9-3.1 µm in diam.

Aspergillus fonsecaeus Thom and Raper.

Synonyms: S. fusca Bainier,in Bul. Soc. Bot. France 27:29, pl.1 fig.5 (1880)

Plate-1-I

Colonies grown on PDA, basal vegetative mycelium largely submerged in more or less conspicuous concentric zones, heads carbon black or brownish black, imparting to the colony alike coloration , reverse colorless in young, conidial heads large , globose, radiate or with chains of conidia maased in an indefinite number of loose divergent columns commonly 150- 300 µm in diam., conidiophores varying in lenth fron 1500-3500×20-30 µm , globose, fertile over the entire, surface, 50-75 µm in diam., brown to dark; sterigmata in two series, brown, primary sterigmata variable in different heads, ranging from 15-20×6-8 small head and 35-45 µm in diam., in large heads, secondary sterigmata 8-14×5-6.5 µm in diam., conidia large, globose, conspicuously roughened with prominent colored bars ,3.1-9.4 µm in diam.

Fusarium mangiferae Britz, M.J. Wingf. And Marasas, Mycologia: 725 (2002)

Plate-1-J

Colonies grown on PDA, white aerial mycelium, floccose, reverse of colonies sometimes rosy buff to dark purple, conidiophores sympodially branched bearing mono- and polyphialides, have 2-5, conidiogenous openings, microconidia variable in shape, obovoid conidia abundant, oval to allantoids conidia occurring, occasionally microconidia.

Plate - 1

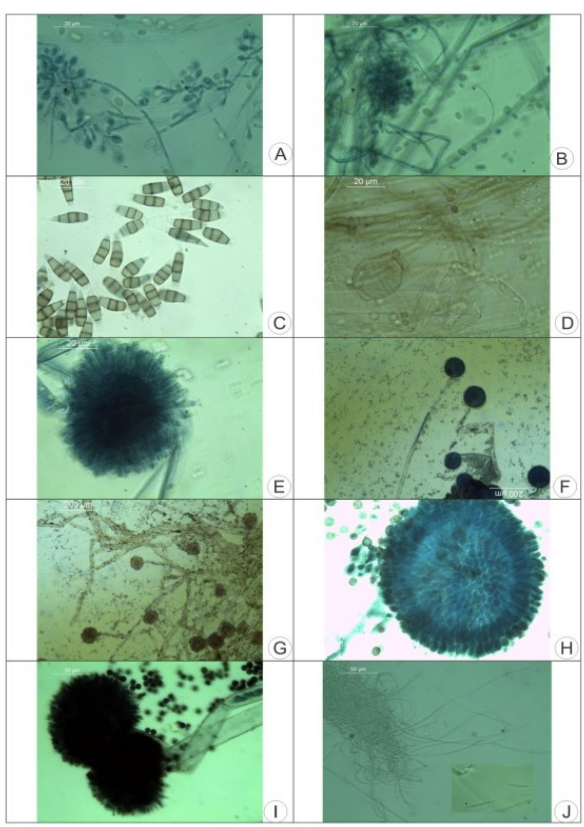

Fig. : A : *Scedosporium prolificans;* B. *Scedosporium apiospermum;* C. *Pestalotiopsis hainanesis;* D. *Mucor cercinelloides;* E. *Sycephalastrum racemosum;* F. *Aspergillus violaceo-fuscus;* G. *Aspergillus japonicus;* H. *Aspergillus niger;* I. *Aspergillus fonsecaeus;* J. *Fusarium mangiferae*

CONCLUSION :

The study carried out for taxonomy of leaf litter fungi from sacred grove of Kolhapur district of Maharashtra. Major contribution of leaf litter fungi occurred into decomposition of forest litter like that of reserved forest of sacred groves. In view of this, the present research conducted to study diversity of leaf litter fungi from this sacred grove may play an important role in decomposition. Such as, 2 sp of *Scedosporium* ; 1 sp of *Pestalotiopsis*;1 sp of *Mucor*, 1 sp of *Syncephalastrum sp;* 4 sp of *Aspergillus* and 1 sp of *Fusarium*.

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ENUMERATION OF WEED PLANTS FROM KADEGAON TEHSIL, SANGLI (MAHARASHTRA), INDIA

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ABSTRACT: *The research work was undertaken to study the weed plants of Kadegoan tehsil, Sangli district Maharashtra. It is positioned at 17.15° N latitude and 74.15° E longitude with average elevation of 271 metres. Submountain ranges of Western Ghat are also present in the tehsil. Sagareshwar Wild Life Sanctuary, Dongarai Valley and Sonsol ghats have a very rich diversity with respect to weed plants. The Kadegaon Tahsil has a moderate climate with hot summer and fluctuating winter and monsoon season. The temperature ranges from 23°C to 45ºC with minimum humidity. The black and red soil, bright sunlight are the two important natural resources abundantly available in this region which are responsible for the development of dry deciduous thorny vegetation.*

The weed plants are responsible for reducing the crop productivity, quality and input efficiency, due to interference in agricultural operations in crop fields. The crop productivity is also hampered as these weed are alternate hosts for some pests and pathogens. These factors lead ultimately towards higher cost for cultivation by multiple folds. Hence proper identification of such weed is required for their proper management. Due to such factors very diverse members of weed families have been documented in the present investigation.

The study reveals that there is variation in weed flora in different seasons. The weeds like Congress, Nirgudi, Dudhi, Dudhani, Bhui awala, Punarnava, Idra-Ichka, Aaghada, Kandisher are common. The enumeration of weed flora cited total 42 families with 118 species. Out of these, 36 families of dicot are represented by 80 genera and 96 species. Moreover 22 monocot species under 21 genera belonging to 6 families have been recorded. The most species rich families were Acanthaceae, Amaranthaceae, Asteraceae, Euporbiaceae, Leguminose, and Poaceae.

Keywords: *Weed, deciduous, taxonomy, species*

INTRODUCTION:

The research work was undertaken to study the weed plants of Kadegoan tehsil, Sangli district, Maharashtra. It is positioned at 17.15° N latitude and 74.15° E longitude with average elevation of 271 metres. Submountain ranges of Western Ghat are also present in the tehsil which include Sagareshwar Wild Life Sanctuary, Dongarai Valley, Chauranginath and Sonsol ghats having very rich diversity with respect to weed plants. The Kadegaon Tehsil has a moderate climate with hot summer and fluctuating winter and monsoon season. The temperature ranges from 23°C to 45ºC with minimum humidity. The black and red soils with bright sunlight are the two important natural resources abundantly available in this region which are responsible for the development of dry deciduous thorny vegetation.

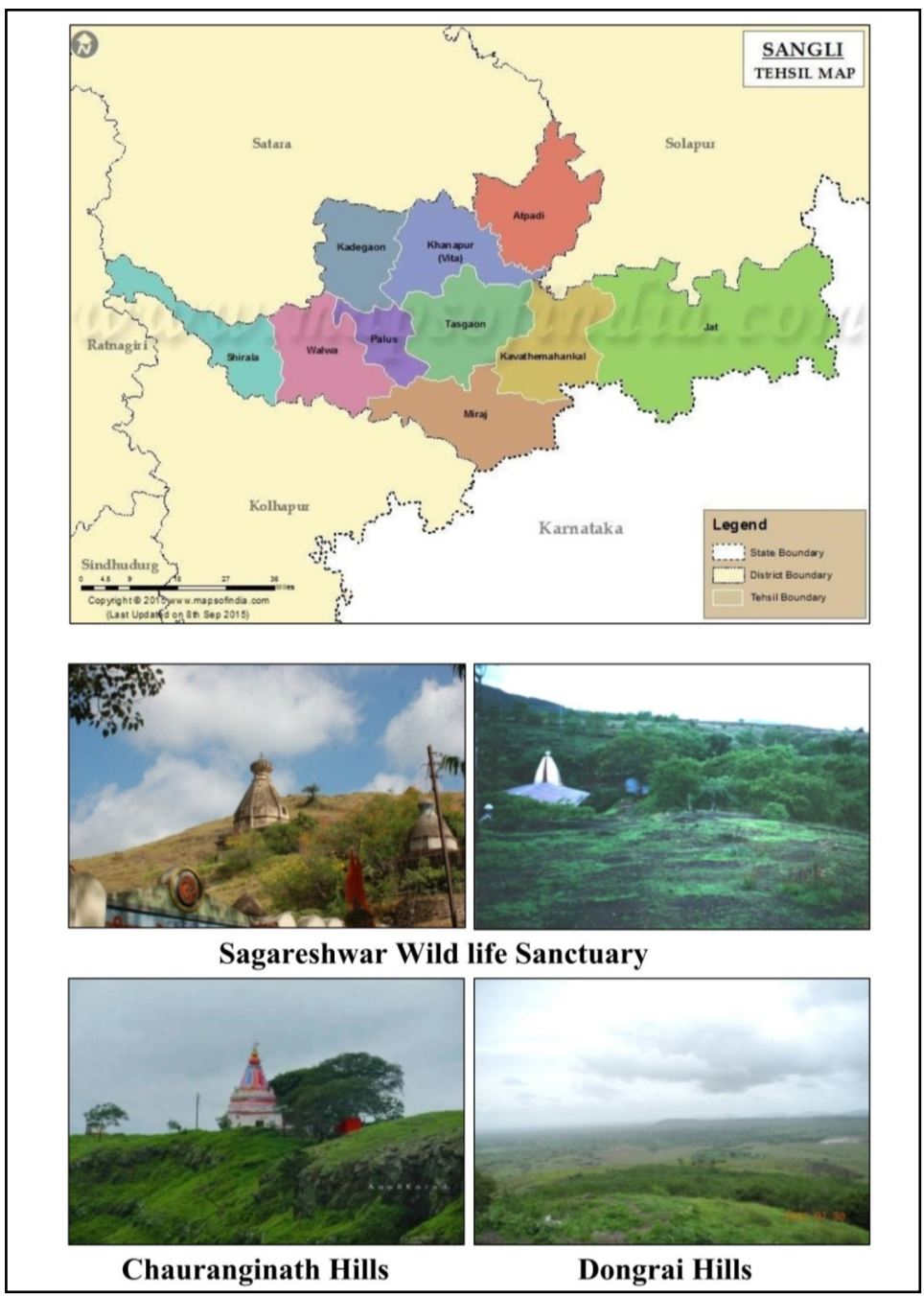
Weeds, in crop field, decrease input efficiency, interfere with agricultural operations, spoils quality and act as alternate hosts for many insect pests and diseases. Some weeds liberate cytotoxic substances that affect the crop growth. The apparent result of these traits is that the hike in rate of cultivation by many folds. Together with this, weeds have an effect and interfere within the management of all the terrestrial and aquatic resources. They endanger the native flora by choking and deliberate takeover them. Knowing the weeds that are competitive with the fascinating crops is very important to grasp the way to manage their populations. It's same that identification is half the way for its management. The primary step in effective weed management is the correct identification for basic understanding of the weeds life cycle. Correct identification is an essential step in ensuring that new weeds are eradicated before they even reproduce. As after reproduction, the seed which are light weighted and can easily be dispersed by different agencies and making them aggressive. Correct weed identification will facilitate in choosing right weedicides to regulate the weed. Also knowing the life cycle of such weed helps in timing of weedicide application. Many agricultural operations are also used for proper weed control.

Recently, Naidu (2012) illustrated weeds with respect to their morphological characters which help in proper identification of such weeds. Gupta (1998, 2004, 2007, 2010) published in his book weed management with reference to fundamental and modern techniques used. Also Awale (2014) has carried out floristic studies of Sangli District and reported 1541 angiosperm plant species belonging to 164 families

of which 1253 are dicotyledons belonging to 135 families and 288 are monocotyledons belonging to 29 families. Salunkhe's (2015) study on biodiversity of Yashwantaro Chavan Sagreshwar Wildlife Sanctuary reports 209 angiosperms belonging to 66 families, 172 genera, 81 birds, 16 reptiles and 11 mammals. Such studies aid in knowing the weeds of this area in relation to the flora. The present study presents a species list of the weed flora recently created, to increase our knowledge of the vegetation growing there and provide subsidies.

STUDY AREA:

Study the weed plants of Kadegoan tehsil, Sangli district Maharashtra This study area lies between $17^{0}15'$ N and $74^{0}15'$ E latitudes and at the height of 271 meters from MSL. It can be considered a dry deciduous forest and exposed to fluctuating environmental condition. The prone area and forest lies in a drought comprise semi deciduous hills. The survey was conducted from different seasons to observe the weed flora.



MATERIAL AND METHOD :

The study area was visited regularly to collect the plant specimens. The observations on ecological aspects along with morphology were made during the visits. The plant specimens were collected in polythene bags and brought to the laboratory. Collected specimens were poisoned with 1% mercuric chloride solution in alcohol as pesticide and later were numbered and mounted on standard herbarium sheet and kept in the department as per method of Naik (1989).

The mounted herbarium sheets were identified using textbook and standard flora's viz. Flora of Bombay Presidency, 3 volumes by Cooke (1901-1914), Flora of Maharashtra by Naik and associates (1989), Bentham G and Hooker JD (1862-1883), Flora of Maharashtra State, Dicotyledons by Singh et. al. (2004), Flora of Maharashtra State, Monocotyledons by Sharma et. al. (1996), Flora of Kolhapur District, S. R. Yadav and M. M. Sardesai (2002),Flora of Baramati by R.B.Bhagat et.al. Flowers of Sahyadri (Ingalhalikar, 2001) and Handbook of Weed Identification by VSGR Naidu (2012) were also referred. Recently published Flora of Sangli District (Awale, 2014) was also used. Authentication of taxanomic names and ranks were done by *The Plant List* **(2013).**

RESULTS AND DISCUSSION:

The research work was undertaken the weed flora of Kadegaon thasil, Dist.-Sangli. These are the most important localities for ecological and biological studies in South-Western hilly region of Maharashtra. During the study of weed plants in this area, the enumeration of weed flora cited in total 42 families with 118 species. Out of these, 36 families of dicot are represented by 80 genera and 96 species. Moreover 22 monocot species under 21 genera belonging to 6 families have been recorded. The most species rich families were Acanthaceae, Amaranthaceae, Asteraceae, Euporbiaceae, Leguminose, and Poaceae.

The study reveals that there is variation in weed flora in different seasons. The weeds like Congress, Nirgudi, Dudhi, Dudhani, Bhui awala, Punarnava, Idra-Ichka, Aaghada, Kandisher were common.

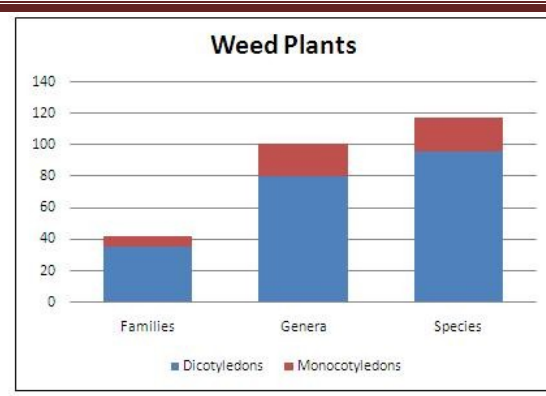
List of Weed Plant:

Sr. No.	Family	Botanical name
1	ACANTHACEAE	*Andrographis paniculata* (Burm.f.) Nees
		Hygrophila auriculata (Schumach.) Heine
		Barleria cristata L.
		Barleria prionitis L.
		Blepharis sp.
		Gomphrena sp.
		Ruellia prostrata Poir.
2	ALISMATACEAE	*Sagittaria* sp
3	AMARANTHACEAE	*Achyranthes aspera* L.
		Alternanthera brasiliana (L.) Kuntze.
		Alternanthera pungens Kunth
		Alternanthera sessilis (L.) R.Br. ex DC.
		Amaranthus spinosus L.
		Amaranthus viridis L.
		Celosia argentea L.
4	APIACEAE	*Centella asiatica* (L.) Urb.
5	APOCYNACEAE	*Catharanthus roseus* (L.) G.Don
6	ASCLEPIADACEAE	*Calotropis gigantea* (L.) Dryand.
		Calotropis procera (Aiton) Dryand.
		Hemidesmus indicus (L.) R. Br. ex Schult.
7	ASTERACEAE	*Acanthospermum hispidum* DC.
		Ageratum conyzoides (L.) L.
		Ageratum houstonianum Mill.
		Bidens pilosa L.

		Blumea oxyodonta DC.
		Emilia sonchifolia (L.) DC. ex DC.
		Erigeron bonariensis L.
		Parthenium hysterophorus L.
		Senecio sp.
		Tridax procumbens (L.) L.
		Xanthium strumarium L.
8	BORAGINACEAE	*Heliotropium indicum* L.
		Trichodesma indicum (L.) Lehm.
9	CANNACEAE	*Canna indica* L.
		Cannabis sativa L.
10	CAPPARIDACEAE	*Cleome gynandra* L.
11	CHENOPODIACEAE	*Chenopodium album* L.
		Chenopodium murale L.
12	COMMELINACEAE	*Commelina diffusa* Burm.f.
		Cyanotis cristata (L.) D. Don.
13	CONVOLVULACEAE	*Convolvulus arvensis* L.
		Evolvulus alsinoides (L.) L.
		Ipomoea carnea Jacq.
		Ipomoea rubriflora O'Donell
14	CUCURBITACEAE	*Diplocyclos sp.*
15	CYPERACEAE	*Cyperus difformis* L.
		Cyperus rotundus L.
		Fimbristylis sp.
		Rhynchospora colorata (L.) H.Pfeiff.
16	EUPHORBIACEAE	*Acalypha indica* L.
		Euphorbia heterophylla L.
		Euphorbia hirta L.
		Euphorbia thymifolia L.
		Euphorbia tirucalli L.
		Phyllanthus niruri L.
17	FABACEAE	*Aeschynomene indica* L.
		Alysicarpus longifolius (Spreng.) Wight & Arn.
		Alysicarpus monilifer (L.) DC.
		Chamaecrista pumila (Lam.) K.Larsen
		Clitoria ternatea L.
		Crotalaria sp.
		Desmodium sp.
		Indigofera linifolia (L.f.) Retz.
		Lathyrus sativus L.
		Mimosa pudica L.
		Senna occidentalis (L.) Link
		Senna tora (L.) Roxb.
		Tephrosia purpurea (L.) Pers.
		Vigna trilobata (L.) Verdc
18	LAMIACEAE	*Leonotis nepetifolia* (L.) R.Br.
		Leucas aspera (Willd.) Link.
		Rotheca serrata (L.) Steane & Mabb.
		Volkameria inermis L.

19	LYTHRACEAE	*Ammannia baccifera* L.
20	MALVACEAE	*Abelmoschus ficulneus* (L.) Wight & Arn.
		Abelmoschus moschatus Medik.
		Abutilon indicum (L.) Sweet.
		Hibiscus sp.
		Sida acuta Burm. f.
		Sida spinosa L.
21	MENISPERMACEAE	*Cocculus hirsutus* (L.) W.Theob.
22	NYCTAGINACEAE	*Boerhavia diffusa* L.
		Boerhavia erecta L.
23	ONAGRACEAE	*Ludwigia sp.*
24	OROBANCHACEAE	*Striga asiatica* (L.) Kuntze
25	OXALIDACEAE	*Biophytum sensitivum* (L.) DC.
		Oxalis corniculata L.
26	PASSIFLORACEAE	*Passiflora edulis* Sims
27	PAPAVARACEAE	*Argemone mexicana* L.
28	PLUMBAGINACEAE	*Plumbago zeylanica* L
29	POACEAE	*Arundo donax* (L.)
		Avena sterilis subsp. ludoviciana (Durieu) Gillet & Magne
		Axonopus sp.
		Brachiaria reptans Gard. & Hubb.
		Chloris sp.
		Coix sp.
		Cynodon dactylon (L.) Pers.
		Digitaria sp.
		Eragrostis sp.
		Heteropogon sp.
		Paspalum sp.
		Setaria sp.
30	POLYGONACEAE	*Antigonon leptopus* Hook. & Arn.
		Persicaria glabra (Willd.) M.Gómez
31	PONTEDERIACEAE	*Eichhornia crassipes* (Mart.) Solms.
32	PORTULACACEAE	*Portulaca oleracea* L.
33	PRIMULACEAE	*Anagallis arvensis* L.
34	SAPINDACEAE	*Cardiospermum halicacabum* L.
35	SCROPHULARIACEAE	*Celsia coromandelina* J.Koenig ex Rottb.
		Sopubia delphinifolia (L.) Don.
		Verbascum chinense (L.) Santapau
36	SOLANACEAE	*Datura metel* L.
		Solanum americanum Mill.
37	TILIACEAE	*Corchorus sp.*
38	TYPHACEAE	*Typha latifolia* L.
39	VERBENACEAE	*Lantana camara* L.
40	VITIACEAE	*Vitex negundo* L.
41	URTICACEAE	*Urtica* sp.
42	ZYGOPHYLLACEAE	*Tribulus terrestris* L.

Total data of the survey			
Class	**Families**	**Genera**	**Species**
Dicotyledons	**36**	**80**	**96**
Monocotyledons	**6**	**21**	**22**
Total	**42**	**101**	**118**



CONCLUSION:

The present investigation reveals high diversity in relation to weeds belonging to 42 families with 118 species. As the temperature and also the topography of the studied area is mostly dry, the flora distributed is mostly dry deciduous thorny type. The weeds play and important role in the survival of such vegetation, as these weed introgress establishment of the natural vegetation. Also these weeds interfere in the agricultural crops and practices leading failure in the crop growth. Finally, they reduce the crop productivity. Hence, it is necessary for proper management of such weeds. Use of weedicides or other fundamental and modern techniques of management are mandatory. The survey will help in proper identification of weed and moreover by studying the life pattern of these weeds, they can be controlled.

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ISOLATION AND IDENTIFICATION OF SOIL FUNGI IN BANANA FIELD AT VITA, SANGLI DISTRICT, MAHARASHTRA, INDIA

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ABSTRACT: *The conservation of diversity of mycoflora in agricultural fields becomes very essential for the development of sustainable agriculture. In the present investigation, sixty five fungal species representing 26 genera were isolated and identified from banana field at Vita, Sangli District the dominant genera in banana field were Aspergillus, Penicillium and Trichoderma species. The present study clearly revealed that the diversity and distribution of soil fungi in agricultural field.*

Keywords: *Diversity, Fungi, banana field*

INTRODUCTION :

Microorganisms in the soil are beneficial in increasing soil fertility and plant growth as they are involved in several biochemical transformations and mineralization activities in soils. Fungi are an important component of soil micro biota more in abundance than bacteria, depending on soil depth and nutrient conditions.

The fungi found in the soil, inhabit the soil as their natural ecological habitat. The term soil fugi is generally applied to the heterogeneous collection of fungi isolated from soil or the fungi which have been observed as growing in the soil. Some of these are unquestionably soil fungi in the most restricted sense & play an important role in the breakdown of organic debris. Other is transient, & is transported by wind, water, or some other agent to an essentially foreign habitat. Some are facultative parasites, rain washed from their hosts or released by slow decay. Association of all soil fungi is not always beneficial to the plant.

The conservation of diversity of fungi in agricultural fields becomes very essential for the development of sustainable agriculture. Banana is an important fruit crop of many tropical and subtropical regions of India. It is cultivated in India in an area of 830.5 thousand ha and total production is around 29,779.91 thousand tons. Main banana growing states are Tamil Nadu, Maharashtra, Gujarat, Andhra Pradesh and Karnataka. Hence in the present investigation was designed to study the soil fungi from banana field of Vita, Sangli District.

MATERIAL AND METHODS :

A) Material – Two type of soil are selected for this study and were designated A &B

Two type soil A & B were taken from Banana fields. Soil A was deposited clay, brownish in colour collected from near Vita ponds. Soil B was black cotton, black in colour collected from Alsund road, Vita.

Both the soils from Banana field were manured with organic manures and watered 10 day interval, except in rainy season. There were some common weeds like *Cynodon dactylon, Cypures rotundus, Digitaria spp. & Parthenium spp.*

B) Collection of soil sample -

Soil sample were collected on the 5th day of every month from January 2018 to December 2018. Collection of soil samples taking composite samples up to a depth of 6 inches, after scraping off an inch of surface soil with a sterile trovel. A pit was dug with the trovel which was sterilized with 70 % alcohol. The soil was collected in polythene bags & was brought to the laboratory.

C) Isolation of the fungal flora from the soil samples -

The soil microfungi were study by dilution plate method (Waksman, 1922).

Soil to be diluted is silted through the sieve with 2mm pores. 1gm of the sieve dry soil was mixed with 100 ml. sterile water in conical flask & thoroughly shake for some time. To obtain 1:1000 dilutions with sterile pipette, pipette out 1 ml. of soil suspension & added to 9 ml. of sterile water in test tube which gives 1:10,000 dilution. From these dilutions pipette 1 ml. sample & place in sterilized petriplates. To these plates add approximately 20ml. of melted but cooled culture media. Then rotated the discs by hand in browed swirling motion, so as to disperses the sample in the agar uniformly. Incubated the plates for 5-6 days at room temperature (25-29.5 ^{o}C), before counting for a fungal colonies. Examined the plates regularly.

D) Culture media –

The fungi were isolated PDA media

Potato dextrose agar (PDA)

Potato	–	200gm.
Dextrose	–	20gm.
Agar	–	15gm .
Distilled water	–	1000ml.
Streptomycin	–	30mg.

Potatoes are peeled and sliced, later boil in a liter of water for one hour. Filtered it through a muslin cloth & make up volume to one liter. Add Agar & Dextrose in filtrate. The medium is autoclaved. Streptomycin is added to the cooled liquid medium before pouring it in petridishes.

E) Transferring fungi to slant –

Some fungi are slow growing. After two to three days some fast growing fungi like *Mucor*, *Rhizopus* contaminated the slow growing fungi and after five to six days conidial stages, thus there was contamination the slow growing fungi and after five to six days conidial stages of *Aspergillus*, *Trichoderma* gives rise to secondary colonies, thus there was contamination. Slow growing fungi were picked up and transferred to the slants. So that pure culture was obtained.

Transfer of the fungi from colonies to the slants was made by using flamed sterilized chrome loop. At the time of the transfer, the test tube containing the slant or organism was held near the flame. This helps in avoiding the contamination during the transfer.

F) Identification –

Identification of the fungi was done with the help of relevant standard keys.

G) Preservation –

For maintaining the pure culture the cultured tubes were kept in refrigerator. Because of reduced temperature, the drying of the tubes is slowed down & subculturing intervals can be increased four months. This is widely used procedure.

H) Method of counting the number of fungi in the soil –

It has been pointed out by Waksman (1922) that in the determination of the number of fungi by dilution plate method is so great, as to makes the results absolutely worthless. To reduce the variability of the number of fungi on the plate and thus obtain a slow probable error, low dilution has to be used so as to have 30 to 100 fungus colonies developing on the plate. This would necessitate a dilution of only 500 to 2000 for an ordinary fertile soil.

Waksman (1922) and other have long made use of the fact that when a culture of a fungus is wanted free from bacteria, raisin agar which is acid in reaction may be used. A medium has, therefore, been devised having a reaction acid enough to prevent the development of actinomycetes and the great majority of bacteria. The following synthetic medium was used.

Agar	-	25 gm.
Glucose	-	10 gm.
KH_2PO_4	-	1 gm.
Peptone	-	5 gm.
$MgSO_4 7H_2O$	-	0.5 gm.
Distilled water	-	1000 ml.

The P^H was adjusted with phosphoric acid to 3.6 & with agar 4.

The soil was diluted in regular manner to only (1:100) to (1:1000). To obtain an accurate count and a low probable error ten plates were prepared for each soil.

The plates were incubated for 72 hours at 25^0 C. The colonies may be counted after 48 hours, then after 72 hours, due to fact that in some soils, rich in mucorales, the spreading forms will tend to overgrow the plate in 72 hours.

RESULT & DISCUSSION :

Table -1 : Distribution of fungi in the two soils

Sr. No.	Fungus	Soil A	Soil B
1	*Rhizopus stolonifer*	P	P
2	*Rhizopus oryzae*	P	P

3	*Rhizopus nodosus*	P	P
4	*Mucor hiemalis*	A	P
5	*Mucor varians*	A	P
6	*Thielavia terricola*	P	P
7	*Chaetomium globosum*	P	A
8	*Phoma humicola*	A	P
9	*Geotrichum candidum*	P	A
10	*Trichoderma viride*	P	P
11	*Aspergillus fumigatus*	P	P
12	*Aspergillus versicolor*	P	A
13	*Aspergillus flavus*	P	P
14	*Aspergillus terreus*	P	A
15	*Aspergillus niger*	P	P
16	*Aspergillus phoenicus*	P	P
17	*Aspergillus candidus*	A	P
18	*Penicillium Link I*	P	A
19	*Penicillium Link II*	A	P
20	*Penicillium Link III*	P	A
21	*Penicillium Link VI*	A	P
22	*Humicola grisea*	P	P
23	*Cladosporium cladosporoides*	P	P
24	*Cladosporium oxysporum*	P	P
25	*Scytalidium lignicola*	P	A
26	*Curvularia lunata*	P	A
27	*Helminthosporium nodulosum*	P	A
28	*Drechslera halodes*	P	A
29	*Fusarium oxysporum*	P	P
30	*Fusarium culmorum*	A	P
31	*Rhizoctoia solani*	A	P
32	*Sclerotium rolfsil*	P	P
33	White mycelium	P	A
34	Black mycelium	P	P

P = Present A = Absent

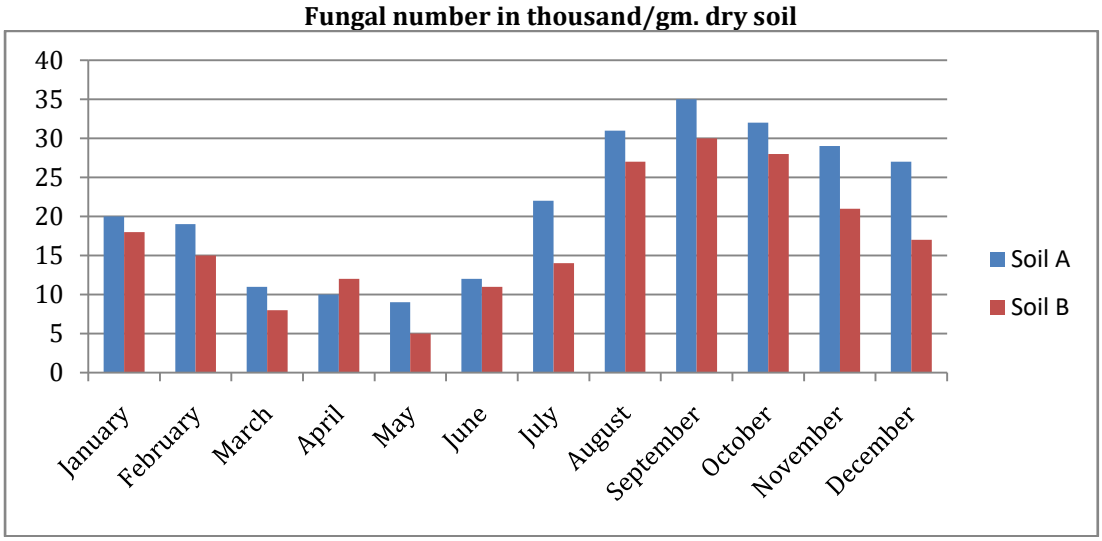
Table 2 : Fungal members in thousand/gm. of dry soil A & B during twelve months.

Sr. No.	Months	Fungal number in thousands/gm. of dry soil	
		Soil A	Soil B
1	January	20	18
2	February	19	15
3	March	11	8
4	April	10	12
5	May	9	5
6	June	12	11
7	July	22	14
8	August	31	27
9	September	35	30
10	October	32	28
11	November	29	21
12	December	27	17

Table -3 : Seasonal variation of soil fungi

Sr. No.	Fungus	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1	*Rhizopus stolonifer*	++	+	+	+	+	+	+++	++++	++++	++++	+++	+++
2	*Rhizopus oryzae*	++	+	+	-	-	++	+++	++++	++++	+++	++	++
3	*Rhizopus nodosus*	+	-	-	-	-	++	++	++	+++	++	+	+
4	*Mucor hiemalis*	-	-	-	-	-	+	+	++	++	+	-	-
5	*Mucor varians*	+	+	-	-	-	++	++	++	+++	+	+	+
6	*Thielavia terricola*	-	+	-	-	-	-	+	-	+	-	-	-
7	*Chaetomium globosum*	-	-	+	-	-	-	-	-	+	-	-	-
8	*Phoma humicola*	-	-	-	-	-	+	-	-	-	-	-	+
9	*Geotrichum candidum*	-	-	-	+	-	-	-	-	-	-	-	-
10	*Trichoderma viride*	-	-	-	-	-	-	++	-	-	+	-	-
11	*Aspergillus fumigatus*	++	++	++	+++	+++	+++	++	+	+	++	+	+
12	*Aspergillus versicolor*	-	-	-	+	-	++	-	-	+	+	-	-
13	*Aspergillus flavus*	++ ++	+	+	+	+	-	-	-	-	-	+	-
14	*Aspergillus terreus*	-	-	+	+	-	-	-	-	-	-	-	+
15	*Aspergillus niger*	++ ++	++ ++	+++ +	+++ +	+++	++ +	++	++	++	+++	++	+
16	*Aspergillus phoenicus*	+	+	+	++	+	++	+	+	+	+	+	+
17	*Aspergillus candidus*	++	+	+	+	+	++	++	+	+	+	+	+
18	*Penicillium Link I*	+	-	-	-	-	-	-	++	-	+	-	-
19	*Penicillium Link II*	-	-	-	-	+	+	-	+	-	++	-	-
20	*Penicillium Link III*	-	-	-	-	-	-	+	+	-	-	-	-
21	*Penicillium Link VI*	+	-	-	-	-	-	-	-	-	-	-	-
22	*Humicola grisea*	-	-	-	-	-	-	-	-	-	-	+	-
23	*Cladosporium cladosporoides*	++ ++	++ +	+	++	+	++	++	+++	++++	+++	++++	+++
24	*Cladosporium oxysporum*	++ ++	++ +	+	+	+	++	++ +	+++	++++	++++	++	++
25	*Scytalidium lignicola*	+	+	-	-	-	-	+	-	-	-	-	-
26	*Curvularia lunata*	+	-	-	-	-	+	-	+	-	-	+	-
27	*Helminthosporium nodulosum*	-	-	-	-	-	-	-	+	-	+	-	-
28	*Drechslera halodes*	-	-	-	-	-	-	+	-	-	+	-	+
29	*Fusarium oxysporum*	+	-	-	-	-	-	-	-	-	-	+	-
30	*Fusarium culmorum*	-	-	-	-	+	+	+	+	+	-	-	-
31	*Rhizoctoia solani*	-	-	-	-	-	-	++	-	-	-	+	-
32	*Sclerotium rolfsil*	+	-	-	-	-	-	+	-	+	-	-	+
33	White mycelium	+	+	+	+	+	+	+	+	++	++	+	++
34	Black mycelium	-	+	-	-	++	++	-	-	-	+	++	-

Maximum = ++++ Medium = +++ Least = ++ Rare = + Absent = -



DISCUSSION :

It is evident from the table no.1 a large number of genera and species of fungi are common to Banana field, in clay and black soils. Of the 34 species isolated 13 are common to the two soils, a few fungi being restricted in their distribution. According to Rao (1988) it is of interest to note that a number of soil borne plant pathogens are isolated. Some species are appeared only sporadically, while other are predominant and were recorded more frequently in all the seasons.

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SEASONAL VARIATION AND DISTRIBUTION OF MICRO FUNGI FROM SUGARCANE FIELD IN VITA REGION

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ABSTRACT: *Soil is a complex ecosystem hold enormous number of living organisms. This study deals with the monthly variations in soil fungal population of traditional sugarcane field in Vita District, Sangli. About 36 different species belonging to Phycomycetes, Ascomycetes and deuteromycetes were isolated by using PDA medium and identified by using standard manual. The dominant species were Aspergillus, Rhizopus, Cladosporium, Fusarium, Trichoderma, Thielavia, Humicola from the sugarcane field soils of Vita in various months. Total fungal organisms 36 species belong to 17 genera were screened from Vita station District sangli.*

Keywords: *Sugarcane field, Biodiversity, Fungal population.*

INTRODUCTION :

Soils are excellent cultural media for the growth of many types of organisms. This includes Bacteria, Fungi, Algae, Actinomycetes & Viruses. In addition, variation lower animal like Protozoons, Warms, Insects, Rotifers, & various Nematodes are also presents. A spoonful of soil contains billion of microorganism. In general the majority of microbial population is found in the upper six to twelve centimetres of the soil & the number decreases with depth. The number & kind of organism found in soil depends upon the nature of soil, depth, season of the year, state of the cultivation, reaction, organic matter, temperature, moisture, aeration etc. Due to the activities of all these microorganisms, the soil forms dynamic ecosystem.

The fungi found in the soil, inhabit the soil as their natural ecological habitat. The term soil fungi are generally applied to the heterogeneous collection of fungi isolated from soil or the fungi which have been observed as growing in the soil. Some of these are unquestionably soil fungi in the most restricted sense & play an important role in the breakdown of organic debris. Other is transient, & is transported by wind, water, or some other agent to an essentially foreign habitat. Some are facultative parasites, rain washed from their hosts or released by slow decay. Association of all soil fungi is not always beneficial to the plant.

Sugar cane is one of the most important cash crops, which is grown over 6.33 lakh hectares in Maharashtra, 74,497 hectares in Sangli district & 11,000 hectares in Vita Tehsil.

MATERIAL AND METHODS :

A) Material – Two type of soil are selected for this study and were designated A &B

Two type soil A & B were taken from Sugarcane fields. Soil A was deposited clay, brownish in colour collected from near Vita ponds. Soil B was black cotton, black in colour collected from Alsund road, Vita.

Both the soils from Sugarcane field were manured with organic manures and watered 10 day interval, except in rainy season. There were some common weeds like *Cynodon dactylon, Cypures rotundus, Digitaria spp. & Parthenium spp.*

B) Collection of soil sample -

Soil sample were collected on the 5th day of every month from January 2018 to December 2018. Collection of soil samples taking composite samples up to a depth of 6 inches, after scraping off an inch of surface soil with a sterile trovel. A pit was dug with the trovel which was sterilized with 70 % alcohol. The soil was collected in polythene bags & was brought to the laboratory.

C) Isolation of the fungal flora from the soil samples -

The soil microfungi were study by dilution plate method (Waksman, 1922).

Soil to be diluted is silted through the sieve with 2mm pores. 1gm of the sieve dry soil was mixed with 100 ml. sterile water in conical flask & thoroughly shake for some time. To obtain 1:1000 dilutions with sterile pipette, pipette out 1 ml. of soil suspension & added to 9 ml. of sterile water in test tube which gives 1:10,000 dilution. From these dilutions pipette 1 ml. sample & place in sterilized petriplates. To these plates add approximately 20ml. of melted but cooled culture media. Then rotated the discs by hand in browed swirling motion, so as to disperses the sample in the agar uniformly. Incubated the plates for 5-6 days at room temperature (25-29.5 ^{o}C), before counting for a fungal colonies. Examined the plates regularly.

D) Culture media –

The fungi were isolated PDA media

Potato dextrose agar (PDA)

Potato	–	200gm.
Dextrose	–	20gm.
Agar	–	15gm .
Distilled water	–	1000ml.
Streptomycin	–	30mg.

Potatoes are peeled and sliced, later boil in a liter of water for one hour. Filtered it through a muslin cloth & make up volume to one liter. Add Agar & Dextrose in filtrate. The medium is autoclaved. Streptomycin is added to the cooled liquid medium before pouring it in petridishes.

E) Transferring fungi to slant –

Some fungi are slow growing. After two to three days some fast growing fungi like *Mucor*, *Rhizopus* contaminated the slow growing fungi and after five to six days conidial stages, thus there was contamination the slow growing fungi and after five to six days conidial stages of *Aspergillus*, *Trichoderma* gives rise to secondary colonies, thus there was contamination. Slow growing fungi were picked up and transferred to the slants. So that pure culture was obtained.

Transfer of the fungi from colonies to the slants was made by using flamed sterilized chrome loop. At the time of the transfer, the test tube containing the slant or organism was held near the flame. This helps in avoiding the contamination during the transfer.

F) Identification –

Identification of the fungi was done with the help of relevant standard keys.

G) Preservation –

For maintaining the pure culture the cultured tubes were kept in refrigerator. Because of reduced temperature, the drying of the tubes is slowed down & sub culturing intervals can be increased four months. This is widely used procedure.

H) Method of counting the number of fungi in the soil –

It has been pointed out by Waksman (1922) that in the determination of the number of fungi by dilution plate method is so great, as to makes the results absolutely worthless. To reduce the variability of the number of fungi on the plate and thus obtain a slow probable error, low dilution has to be used so as to have 30 to 100 fungus colonies developing on the plate. This would necessitate a dilution of only 500 to 2000 for an ordinary fertile soil.

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Peptone	-	5 gm.
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Distilled water	-	1000 ml.

The P^H was adjusted with phosphoric acid to 3.6 & with agar 4.

The soil was diluted in regular manner to only (1:100) to (1:1000). To obtain an accurate count and a low probable error ten plates were prepared for each soil.

The plates were incubated for 72 hours at 25^0 C. The colonies may be counted after 48 hours, then after 72 hours, due to fact that in some soils, rich in mucorales, the spreading forms will tend to overgrow the plate in 72 hours.

RESULT & DISCUSSION :

Table -1 : Distribution of fungi in the two soils

Sr. No.	Fungus	Soil A	Soil B
1	*Rhizopus stolonifer*	P	P
2	*Rhizopus oryzae*	P	P
3	*Rhizopus nodosus*	P	P

4	*Mucar hiemalis*	P	A
5	*Mucor varians*	P	P
6	*Thielavia terricola*	P	P
7	*Chaetomium spirale*	A	P
8	*Chaetomium globosum*	A	P
9	*Phoma humicola*	P	A
10	*Trichoderma viride*	P	P
11	*Aspergillus fumigatus*	P	P
12	*Aspergillus versicolor*	P	P
13	*Aspergillus flavus*	P	P
14	*Aspergillus terreus*	P	A
15	*Aspergillus niger*	P	P
16	*Aspergillus phoenicus*	P	P
17	*Aspergillus candidus*	P	P
18	*Penicillium Link I*	P	A
19	*Penicillium Link II*	A	P
20	*Penicillium Link III*	A	P
21	*Penicillium Link VI*	P	A
22	*Gliocladium roseum*	A	P
23	*Nigrospora sphaerica*	P	A
24	*Humicola grisea*	P	P
25	*Cladosporium cladosporoides*	P	P
26	*Cladosporium oxysporum*	P	P
27	*Scytalidium lignicola*	P	P
28	*Curvularia lunata*	P	A
29	*Helminthosporium nodulosum*	P	A
30	*Drechslera halodes*	P	P
31	*Alternaria fasiculata*	P	P
32	*Fusarium oxysporum*	P	P
33	*Fusarium culmorum*	P	A
34	*Rhizoctoia solani*	A	P
35	Yellow mycelium	P	A
36	Green Mycelium	P	A

P = Present A = Absent

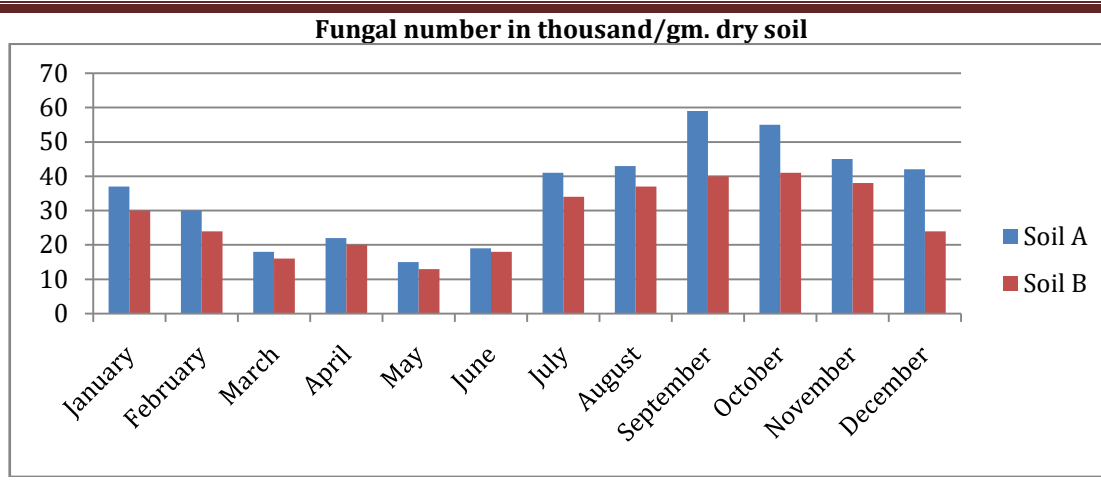
Table 2 : Fungal members in thousand/gm. of dry soil A & B during twelve months.

Sr. No.	Months	Fungal number in thousands/gm. of dry soil	
		Soil A	Soil B
1	January	37	30
2	February	30	24
3	March	18	16
4	April	22	20
5	May	15	13
6	June	19	18
7	July	41	34
8	August	43	37
9	September	59	40
10	October	55	41
11	November	45	38
12	December	42	24

Table -3 : Seasonal variation of soil fungi

Sr. No.	Fungus	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1	*Rhizopus stolonifer*	++	+	+	+	+	+	+++	++++	++++	++++	+++	+++
2	*Rhizopus oryzae*	++	+	+	-	-	++	+++	++++	++++	+++	++	++
3	*Rhizopus nodosus*	+	-	-	-	-	++	++	++	+++	++	+	+
4	*Mucar hiemalis*	-	-	-	-	-	+	+	++	++	+	-	-
5	*Mucor varians*	+	+	-	-	-	++	++	++	+++	+	+	+
6	*Thielavia terricola*	-	+	-	-	-	-	+	-	+	-	-	-
7	*Chaetomium spirale*	-	-	-	-	-	-	-	-	-	-	+	-
8	*Chaetomium globosum*	-	-	+	-	-	-	-	-	+	-	-	-
9	*Phoma humicola*	-	-	-	-	-	+	-	-	-	-	-	+
10	*Trichoderma viride*	-	-	-	-	-	-	++	-	-	+	-	-
11	*Aspergillus fumigatus*	++	++	++	++++	+++	+++	++	+	+	++	+	+
12	*Aspergillus versicolor*	-	-	-	+	-	++	-	-	+	+	-	-
13	*Aspergillus flavus*	++++	+	+	+	+	-	-	-	-	-	+	-
14	*Aspergillus terreus*	-	-	+	+	-	-	-	-	-	-	-	+
15	*Aspergillus niger*	++++	++++	++++	++++	+++	+++	++	++	++	+++	++	+
16	*Aspergillus phoenicus*	+	+	+	++	+	++	+	+	+	+	+	+
17	*Aspergillus candidus*	++	+	+	+	+	++	++	+	+	+	+	+
18	*Penicillium Link I*	+	-	-	-	-	-	-	++	-	+	-	-
19	*Penicillium Link II*	-	-	-	-	+	+	-	+	-	-	-	-
20	*Penicillium Link III*	-	-	-	-	-	-	+	+	-	-	-	-
21	*Penicillium Link VI*	+	-	-	-	-	-	-	-	-	-	-	-
22	*Gliocladium roseum*	+	-	-	-	-	-	-	-	-	-	+	-
23	*Nigrospora sphaerica*	-	+	-	-	-	-	-	-	+	-	-	+
24	*Humicola grisea*	-	-	-	-	-	-	-	-	-	-	+	-
25	*Cladosporium cladosporoides*	++++	++	+	++	+	++	++	+++	++++	+++	++++	+++
26	*Cladosporium oxysporum*	++++	+++	+	+	+	++	+++	+++	++++	++++	++	++
27	*Scytalidium lignicola*	+	+	-	-	-	-	+	-	-	-	-	-
28	*Curvularia lunata*	+	-	-	-	-	+	-	+	-	-	+	-
29	*Helminthosporium nodulosum*	-	-	-	-	-	-	-	+	-	+	-	-
30	*Drechslera halodes*	-	-	-	-	-	-	+	-	-	+	-	+
31	*Alternaria fasiculata*	+	-	-	-	-	-	-	-	-	-	+	-
32	*Fusarium oxysporum*	-	-	-	+	-	+	+	+	+	-	-	-
33	*Fusarium culmorum*	-	-	-	-	+	+	+	+	+	-	-	-
34	*Rhizoctoia solani*	-	-	-	-	-	-	++	-	-	-	+	-
35	Yellow mycelium	-	-	-	-	-	-	+	-	+	-	-	-
36	Green Mycelium	-	-	-	-	+	-	+	-	-	-	-	+

Maximum = ++++ Medium = +++ Least = ++ Rare = + Absent = -



DISCUSSION :

It is evident from the table no.1 a large number of genera and species of fungi are common to Sugarcane field, in clay and black soils. Of the 36 species isolated 13 are common to the two soils, a few fungi being restricted in their distribution. According to Rao (1988) it is of interest to note that a number of soil borne plant pathogens are isolated. Some species are appeared only sporadically, while other is predominant and were recorded more frequently in all the seasons.

The present study confirms the generally accepted view that the commonest soil fungi are representatives of *Aspergillus, Rhizopus, Cladosporium, Fusarium, Trichoderma, Thielavia, Humicola*. It also confirms that Aspergilli dominate in tropical soils. The order of occurrence of the chief genera was *Aspergillus, Rhizopus, Cladosporium, Fusarium, Trichoderma, Thielavia, Humicola sp*.

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PHYSIOLOGICAL STATUS OF MEDICINALY IMPORTANT EPIPHYTIC FERNS FROM MAHABALESHWAR AND PANCHGANI

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ABSTRACT: *The measurement of photosynthetic pigment can provide basic information on the physiological status of plants the quantification of chlorophyll provides important information about the effects of environments on plant growth. In the present study total chlorophyll and carotene content of Asplenium decurrence, Lepisorus nudus and Microsorum membranecium epiphytic ferns have been recorded. The quantitative analysis of chlorophyll, carotene content between vegetative and reproductive leaves shows significant differences between the two stages. In this three fern showed total chlorophyll content was higher in reproductive stage than vegetative stage except Microsorum membranecium. In all three species carotene content was higher in reproductive stage than vegetative stage .The changes in chlorophyll and carotene content in the different species may be due to different microclimatic conditions in study area and related to the medicinal values which play important role in plant physiology.*

Keywords: *Epiphytic Ferns, Photosynthetic pigments, Medicinal value*

INTRODUCTION:

Photosynthesis is one of the most important biochemical processes for plant which convert light energy into stored chemical energy in the form of ATP and NADPH.Chlorophyll is important pigment for physiological process in plants (Richardson et al., 2002, Giletelson 2003). The quantificationofchlorophyll provides important information about the effects of environments on plant growth (Schlemmer et al., 2005) Most pteridophytes live in moist and shady environments (Aldasoro et al., 2004 Karstetd 2005).The measurement of photosynthetic pigment can provide basic information on the physiological status of plants.Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of the leaves, stem, flowers, and roots (Mirza et al., 2013 Srichaikal et al., 2011). The chlorophyll content has medicinal values and also play important role in plant physiology and it can be as nutrition in decline blood sugar condition, digestion, excretion and decreasing allergens (Srichaikal et al., 2011 Singh et al., 2011). The next photosynthetic pigment is carotenoids which are 40 carbon molecules formed by joining 8-c5 isopropane units and can be classified as carotenes, hydrocarbons or xanthophyll. Carotenoides shows extensive conjugated double bonds, making them susceptible to oxidation and free radical reactions (Britton G. et al., 1993).The present study was aimed to reveal the estimation of photosynthetic pigmentprofile and chl.a/b ratio of *Aspleniumdecurrence, Lepisorusnudus, Microsorummembranecium.*

MATERIAL AND METHODS:

The healthy leaves of plant material were collected from different areas of Mahabaleshwar and Panchgani hill region.The chlorophyll were estimated by Arnon (1949) method. One gm of leaf was cut and mixed with clean mortle and pestle. The material were grined gently with addition of 20 ml of 80 %acetone and 0.5gm of MgCo3 powder .Then sample was centrifuged at 5000 rpm for 5 min. and supernatant was transfered to 100 ml volumetric flask.The final volume was made up to 100 ml with addition of 80 % acetone. Then read the absorbance of solution by spectrophotometer using 645 and 663 nm wavelength. Acetone (80%) was used as blank.chlorophyll were calculated by using the formula

Chlorophyll a= 12.7*A663-2.69*A645=X
Chlorophyll b = 22.9*A645-4.68*A663=Y
Total (a+b)= 8.02*A663+20.2*A645=Z
Total chlorophyll=X or Y or Z *Volume of extract /1000*Weight of plant material*100

Carotenoides were estimated by Kirk and Allen (1965) by using the same extract prepared for estimation of chlorophylls. The absorbance was read at 480 nm using spectrophotometer Total carotenoids were calculated by using the formula of Jenson and Jenson (1951).C=D*V*F*10/2500

Where C= total carotenoids in mg g -1 fresh material D=optical density V =total volume in ml
F= dilution factor and 2500= average extinction.

Table no. 1: (Chl. a, chl. b, Total chlorophyll)

Sr. No	Plant name	Chl.a (Veg.stage)	Chl.b (Veg.stage)	Total chl.content (Veg.stage)	Chl.A (Rep.stage)	Chl b. (Rep.stage)	Total chl.content (Rep. stage)
1)	*Asplenium decurrence*	99.06±0.75	80.3±1.7	178.8±0.5	103±1.1	110.6 ± 2	213 ± 1
2)	*Lepisorus nudus*	79.2 ± 1.4	67.03 ± o.5	146 ± 2	79.2 ± 1.4	78.5 ± 2.6	173.3 ± 1.8
3)	*Microsorum membranecium*	93.9 ± 0.7	61.8 ± 0.9	155.3 ± 1.5	83.6 ± 0.7	59.8 ± 0.9	142.6 ± 1.5

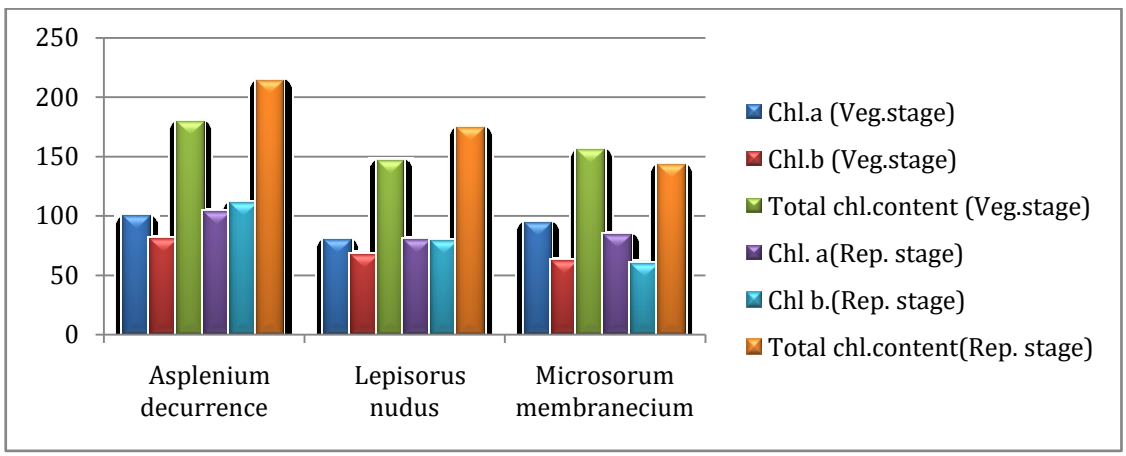
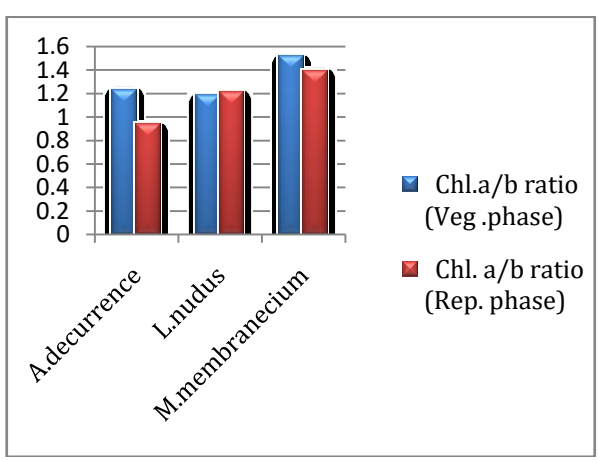
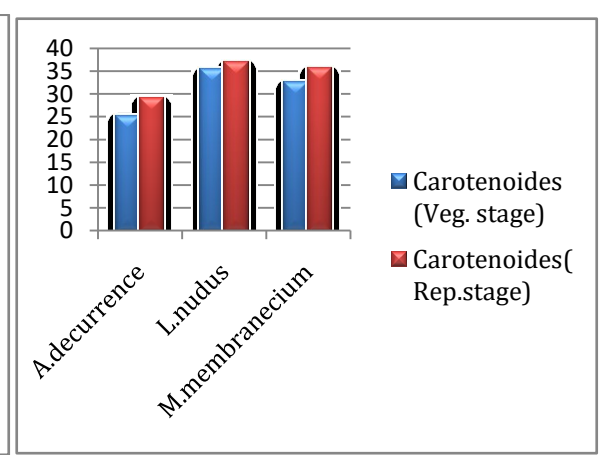


Table 2 : a/b ratio of chlorophyll and Carotenoides

Sr. No	Plant Name	Chl.a/b ratio (Veg .phase)	a/b ratio (Rep. phase)	Carotenoides (Veg. stage)	Carotenoides (Rep.stage)
1.	*Asplenium decurrence*	1.23	0.93	25.24±0.70	28.92±0.84
2.	*Lepisorus nudus*	1.18	1.21	35.44±0.55	37±0.46
3.	*Microsorum membranecium*	1.51	1.39	32.49±0.96	35.61±0.41





RESULT AND DISCUSSION:

In present investigation the values of chl.a, chl.b, total chlorophyll andcarotene contents in the vegetative and reproductive fronds of ferns species are recorded. It is clear from fig 1 the amount of total chlorophyllare higher in the leaves of reproductive stages than vegetative stage. except *Microsorum membranecium.* The higher amount of chl. a and chl. b was found in the leaves of both vegetative and reproductive stages of *Asplenium decurrence.*Vyas and Sharma (1988) who showed that more amount of chl.a than chl. b in *Marsilea aegyptica, Cyclosorus dentatus, Pteris vittata,Tectariacoadunate, Adiantum*

incisum, A.capillusveneris, .Rathorand Sharma(1991) and Sharma et al.,(1995) found the higher contents of chl.a than chl. b in *Isoetes* species. Shakil and Dongare (2008) observed that variation in chlorophyll content may be due to differences in environmental factors like altitude, rainfall, temperature, light intensity and humidity.

In present study showed the highest amount of carotenoids are found in leaves of both vegetative and reproductive stages of *Lepisorus nudus*.Krinsky,(1966) evaluated that during stress conditionthe carotenoids shows sufficient degradation He further indicated that the carotenoids protect the plants from photosynthesized oxidation and chlorophyll degradation .Bohra et al.,(1979) demonstrated that the species of ferns possessing higher carotenoids show less chlorophyll degradation .Kale (2008) showed higher amount of carotenoide contents in the leaves of vegetative phase than reproductive phase. Shakil (2010) recorded higher level of carotenoids in reproductive stage of A. Philippines during winter and summer season. The lower concentrations of carotenoids may be assign to higher concentrations of total chlorophylls evidently low degradation of chlorophylls. In the present investigations, in reproductive stage contain higher amount of carotenoids than the vegetative stage of all the investigated species. Differentiation of carotenoids from specs to species found to be independent of ecological and morphological conditions of plants. The chlorophyll a/b ratio shows lowest values in the leaves of reproductive stage of *Asplenium decurrence* and highest in vegetative stage of *Microsorum membranecium.*The increase in chlorophyll a/b ratio can be assigned to more reduction of chlorophyll -a than chlorophyll –b.The lower values of chlorophyll a/b ratio in the leaves of reproductive stage of *Microsorum membranecium* can be assigned to more reduction of chlorophyll -b content.

CONCLUSION:

The present investigation that in two different species of fern shows chl.a chl.b and total chlorophyll are more in reproductive stage than vegetative stage The variation of content is due to difference in environmental factors. One species shows amount of photosynthetic pigment are higher in vegetative stage than reproductive stage. In this case decrease the level of photosynthetic pigment in reproductive stage due to sporangium formation which creates stress.

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CAMPTOMERIS CASSIAE SP. NOV. ON *CASSIA SOPHERA* L. AND *CASSIA AURICULATA* L. FROM INDIA

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ABSTRACT: *An interesting Dematiaceous Hyphomycetous new species belonging to the genus Camptomeris H. Syd. is illustrate and described. The new species has been recorded on the living leaves of Cassia sophera Linn. and Cassia auriculata Linn. (Fam.: Caesalpiniaceae).*

Keywords: *Mycotaxonomy, Hyphomycetes, Dimatiaceae, new species.*

INTRODUCTION :

During the study of Mycotaxonomy from Southern Kolhapur district and its neighbouring areas, the authors came across an interesting collection on the living leaves of *Cassia sophera* Linn. and *Cassia auriculata* Linn. (Fam.: Caesalpiniaceae). The Dematiaceous Hyphomycetous fungal genus *Camptomeris* H. Syd. is known by 16 valid species and reported mostly on the members of the family Leguminosae from different countries including India. In India three species of *Camptomeris* reported on host genera viz. *Acacia*, *Albizzia* (Fam.: Mimosaceae) and *Crataeva* (Fam.: Capparidaceae), which appears to be doubtful. In present collection the conidia are strictly three septate and are somewhat larger as compared to the existing species of the genus *Camptomeris*. In addition, there is no report of *Camptomeris* on the host genus *Cassia* therefore, a new species has been raised namely *Camptomeris cassiae* sp. nov.

RESULT AND DISCUSSION :

***Camptomeris cassiae* T. R. Kavale et M. S. Patil sp. nov.**

Colonia hypophyllae co sporodochium, phaeo-brunneus ad melano; mycelium immergo; stroma turgidus, unicellularis, vesiculous, 30-46 x 23-28 µm; conidiophorum macronematous, introrsus curvus, inopaniculatus, brunneus, glabro-tunicatus, 48-59 x 3-5 µm; conidium solitarius, aridus, acrogenus, obclavatus vel oblongatus, crassitunicatus, sub-oliaceous-brunneus, verruculosus, tri-septatus, 52-81 x 11-15 µm.

HOLOTYPUS: In foliis vivis *Cassia sophera* Linn. (Fam.: Caesalpinaceae), Dewarde, (Tal.Ajara, Dist.-Kolhapur, M.S.), 9-1-2000, T.R.Kavale, et dipositus, H.C.I.O.-46905, W.I.F. Nos. 2017 ; In foliis vivis *Cassia auriculata* Linn. (Fam.: Caesalpinaceae), Uttur (Tal.-Ajara, Dist.-Kolhapur, M.S.), 1-12-2001, T. R. Kavale, W.I.F.-2018,

Colonies hypophyllous with sporodochia, dark-brown to black; mycelium immersed; stroma with a single large swollen cell and vesicle like, 30-46 x 23-28 µm; conidiophores macronematous, curved inwards, unbranched, brown, smooth, 48-59 x 3-5 µm; conidia solitary, dry, acrogenous, obclavte or oblong, thick-walled, pale olivaceous-brown, verruculose, 3-septate, 52-81 x 11-15 µm.

HABIT: On the leaflets of *Cassia sophera* Linn. (Fam.: Caesalpinaceae), Dewarde (Tal.-Ajara, Dist.-Kolhapur, M.S.), 9-1-2000, T.R.Kavale, H.C.I.O.-46905, W.I.F. Nos. 2017; *Cassia auriculata* Linn. (Fam.: Caesalpinaceae), Uttur (Tal.-Ajara, Dist.-Kolhapur, M.S.), 1-12-2001, T. R. Kavale, W.I.F.-2018.

CONCLUSION :

The existing species of the genus *Camptomeris* H. Syd. are reported mostly on the members of the family Leguminosae from Sri Lanka, Ghana, Pakistan, San Domingo, Sierra Leone, Sudan, Uganda, Cuba, Jamaica, America, Venezuela, Japan and India. However three additional species viz. *C. acaciae* (Syd.) Cif., *C. albizziicola* (Thirum. and Narasimhan) Bessey and *C. crataeva* Subram. are reported from the India on the host genera viz. *Acacia*, *Albizzia* (Fam.: Mimosaceae) and *Crataeva* (Fam.: Capparidaceae). But Ellis, M.B., who considered *Camptomeris* on *Acacia* and *Albizzia,* belongs to *C.albiziae* (Petch) Mason. So these three species from India appears to be doubtful. Present material collected on two species of *Cassia* viz. *C. sophera* Linn. and *C. auriculata* Linn. appears to be quite distinct in respect of strictly three septate conidia and conidia are somewhat larger as to compare the existing species of the genus *Camptomeris*. In addition, there

is no report of *Camptomeris* on the host genus *Cassia* therefore, a new species has been raised namely *C. cassiae* sp. nov. based on the above features.

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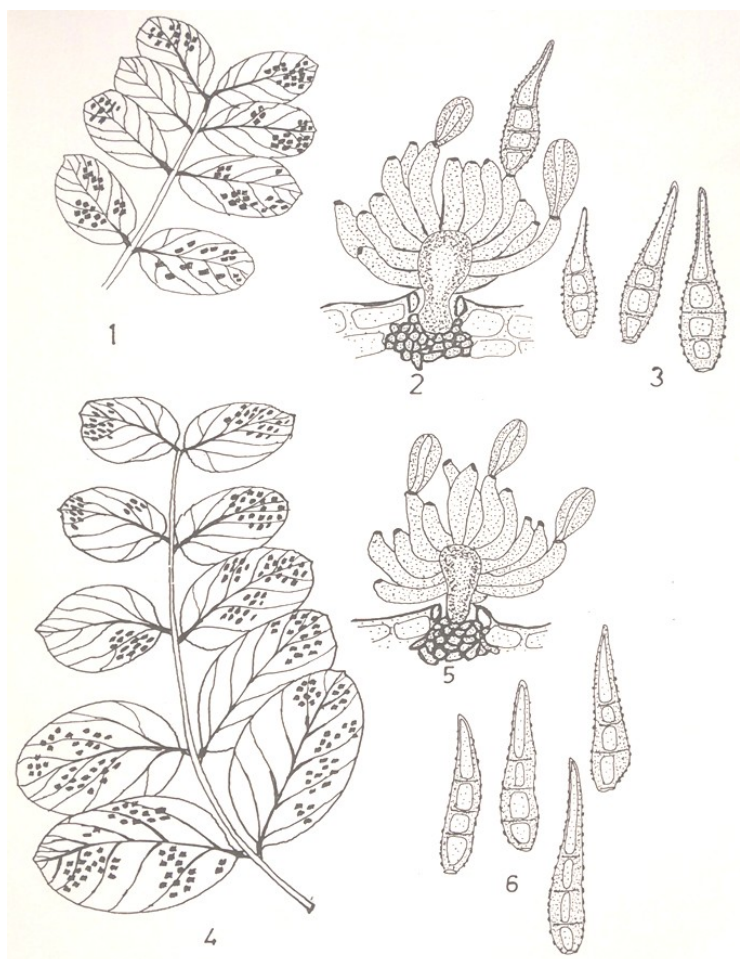

Figs. *Camptomeris cassiae* sp. nov. (figs. 1-3) on the leaves of *Cassia auriculata* Linn. **1.** Habit-infected leaflets showing colonies on lower side; **2.** T. S. of infected leaflets showing sporodichium with conidiophores with young and mature conidia X 360; **3.** Three septate, verrucose conidia X 520. (figs. 4-6) on the leaves of *Cassia sophera* Linn. **4.** Habit-infected leaflets showing colonies on lower side; **5.** T. S. of infected leaflets showing sporodichium with conidiophores with young and mature conidia X 380; **6.** Three septate, verrucose conidia X 555.

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EFFECT OF BIOFERTILIZERS ON PHENOLOGY OF MAIZE *(ZEA MAYS L.)* VARIETY AFRICAN TALL

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ABSTRACT: *An attempt has been made to study the effect of Azotobacter and phosphate solubilizing bacteria (PSB) on Phenology of Zea mays L. (variety –African Tall) at farmland of Santoshwadi, Belanki Dist.Sangli, Maharashtra. The experiment was carried out in a randomized complete block design with three replications. The phenological parameters like plant height, number of leaves per plant, length of leaves, stem and cob diameter and length of cob are measured. It is revealed from the experiment that, there is considerable enhancement in Phenological parameters. The value of 'treatment means' were compared using least significance difference ($p<0.05$).It is evident from the results that biofertilizer treatment producing is found to be high productivity in maize variety African Tall.*

Keywords: *Maize (African tall), Phenology*

INTRODUCTION :

Maize (*Zea mays* L.) is most important cereal crop after wheat and rice. Every part of the maize plant has economic value grain, leaves, stalk, tassel and cob can be used as produce large variety of food and non food production (IITA, 2006). The maize used for human food and animal fodder. Selected and applied methods of biofertilizer increasing integration in production and also coexist environment free from pollution. Apart from this, corn is an important industrial raw material and provides large opportunity (Paroda, 2000).Maize is a C4 mode of carbon fixation plant efficiently utilizes inputs because of its rapid growth and high biomass (Miller *et al*.2010). Beyranvand *et al* 2013 suggested that, the effect of nitrogen and phosphate biofertilizers were evaluated positively, there were an increase in plant height, ear weight, ear length and grain yield. The productivity of maize is dependent on its nutrient requirement and management particularly that of nitrogen, phosphorus and potassium (Arunkumar, 2007). The extensive research programme over the years on beneficial bacteria and fungi has resulted in the development of a wide range biofertilizer which not only fulfill the nutrient requirement of various crop species but increase the crop yield and nutrient composition. Azotobacter species besides playing a role in nitrogen fixation, it has the capacity to synthesize and secrete considerable amounts of biological active substances like vitamins, gibberellins and auxins (Suhag, 2016)

MATERIALS AND METHODS :

To evaluate effect of biofertilizers (*Azotobacter* and *Phosphate Solubilizing Bacteria*) on Phenology of maize (*Zea mays* L.) variety African Tall selected.

Experimental site: The field experiment were conducted at the Experimental farm Belanki (Santoshwadi Lat. $16^0 50'42''$N, Long.$74^0 51'54''$ E), Dist.Sangli Maharashtra.

Experimental design, planting and Fertilizer application: The treatment of bio-fertilizer levels were corresponding to (TA_1), (TA_2), (TA_3), (TP_1), (TP_2), (TP_3) and (TA+P1), (TA+P2), (TA+P3) respectively. The experiments were carried out in plot based on a randomized complete block design (RCBD) with three replications. These doses were applied to the plants at sowing and then together with irrigation every 10-12 days. Each cultivar was planted in 4 ridges, 70 cm between ridges and 20 cm between holes. Seeds rate was maintained at 2 seeds per hole, the seeds were sown manually. Weeding was done manually whenever needed.

Phenological Measurements: At harvest, the following characters were measured: Height of Plant (cm), Number of leaves per plant, Length of Leaves (cm), Stem diameter (cm), cob diameter (cm), Cob length (cm).

Statistical analysis: The collected data was statistically analyzed separately according to the analysis of variance (ANOVA) by and Duncan's Multiple Range Test (DMRT) used to determine the level of significance at $p \leq 0.05$ with SPSS excels software.

Table1: Effect of Biofertilizers on morphological and yield components of Maize (*Zea mays* L.) Variety African Tall

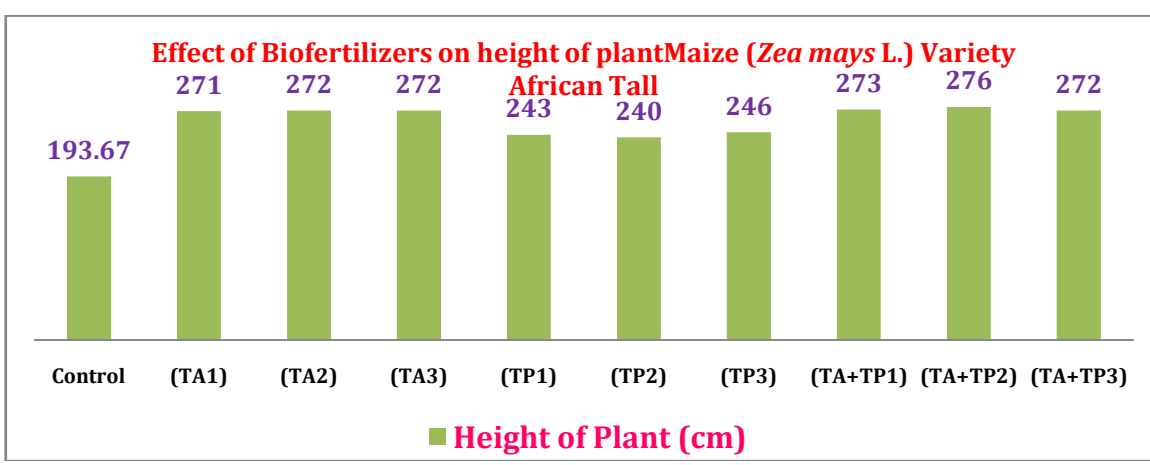
Sr. no	Treatments	Height of Plant (cm)	No.of leaves /plant	Length of leaves	Stem dia-meter (cm)	No. of cob per plant	Length of cob (cm)	Dia-meter of cob(cm)	Horizontal cob lines/ cob	Vertical cob lines/ cob
0	**Control**	193.67e	12	45.00e	2.60e	2	14.67e	3.5e	32c	11e
1	**(TA_1)**	271.00b	14	84.67b	2.77c	2	16.00c	4.3b	38b	15b
2	**(TA_2)**	272.00b	15	74.33c	2.77c	2	16.27b	4.5b	38b	15b
3	**(TA_3)**	272.00b	15	88.00b	2.77c	2	16.17b	4.5b	39b	15b
4	**(TP_1)**	243.00c	13	70.33d	2.73d	2	16.00c	4.1d	35c	13d
5	**(TP_2)**	240.00d	13	71.67c	2.70d	2	15.80d	4.1c	34d	13d
6	**(TP_3)**	246.00c	13	69.33d	2.73d	2	16.00c	4.1c	36c	14c
7	($TA+TP_1$)	273.00b	16	91.33a	2.90b	2	17.33a	4.9a	39b	15b
8	($TA+TP_2$)	276.00a	15	89.67b	2.97a	2	17.33a	4.9a	40a	16a
9	($TA+TP_3$)	272.00b	16	94.00a	2.90b	2	17.33a	4.9a	40a	16a

Different letters (a-e) followed values in same column indicate significant difference in Means of at ($p \leq 0.05$)

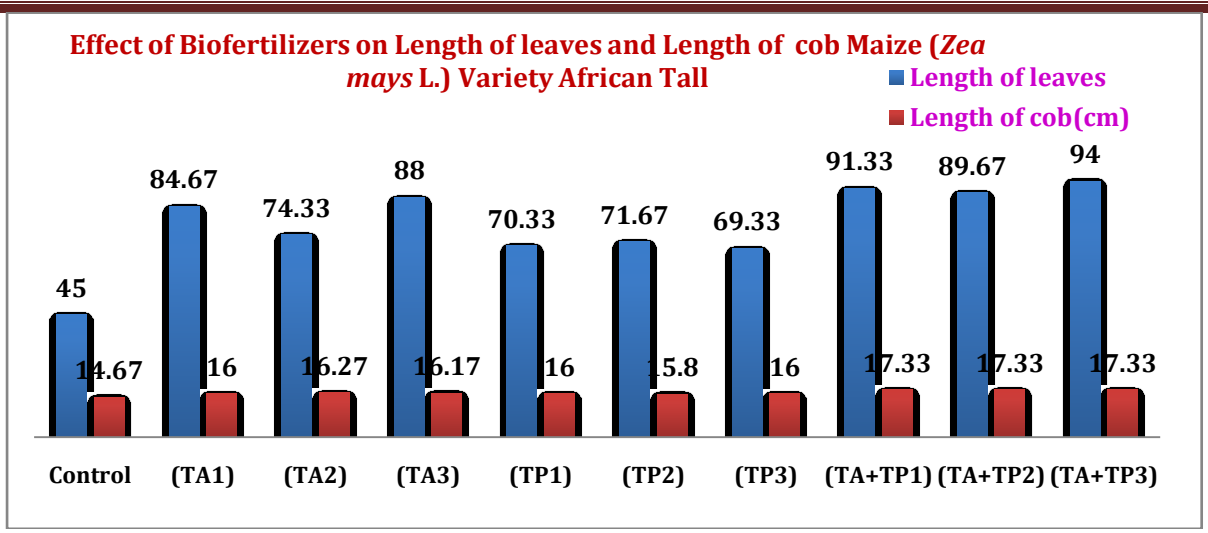
RESULT AND DISCUSSION :

In this study, the results of the effect of biofertilizer on morphological and yield characters of *Zea mays* L. variety Eco-92 were significant shown in Table 1.The application of biofertilizers alone or in combination increased the growth parameters of maize in terms of plant height, number of leaves/plant, leaf length, stem base diameter as well as cob length and diameter. The ANOVA showed significant difference in treated with biofertilizer as compared with control.

Plant height – The Analysis of variance showed that, the effect of biofertilizer on plant height in maize showed a significance increase in height of *Zea mays* L. The highest growth in plant height (276.00a) was recorded with the combination of the biofertilizers (Azotobacter+PSB- $TA+TP_1$) while the lowest growth of plant was recorded in the control (193.67e) Table 1.

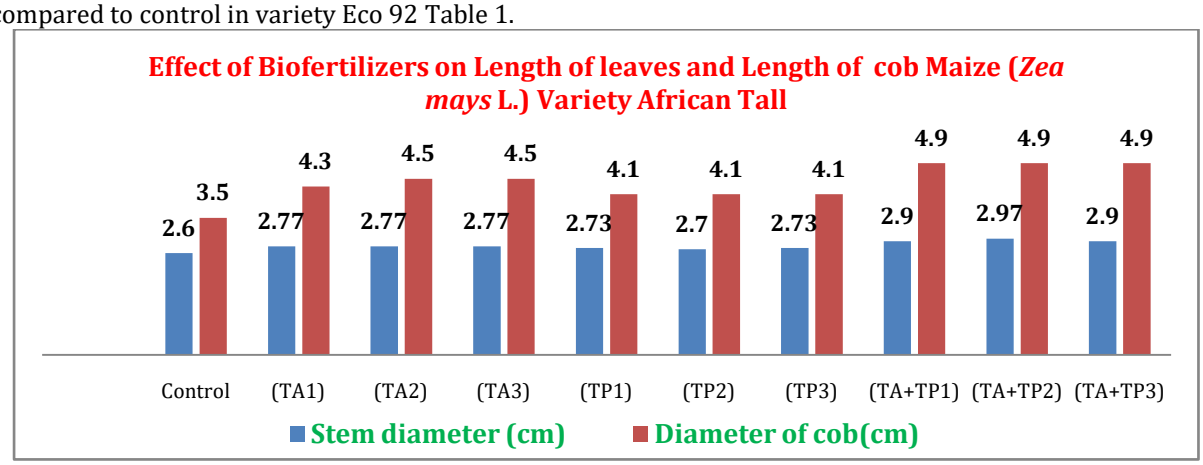


Leaf length - The Analysis of variance showed that, the effect of biofertilizer on length of leaves in maize showed a significance increase in length of *Zea mays* L. The highest length (94.00a) was recorded with the combination of the biofertilizers (Azotobacter+PSB- $TA+TP_3$) while the lowest growth was recorded in the control (45.00e) Table 1.



Stem diameter - The Analysis of variance showed that, the effect of biofertilizer on stem base diameter in maize showed a significance increase in stem diameter of *Zea mays* L. The highest radial growth (2.97a) was recorded with the combination of the biofertilizers (Azotobacter+PSB- TA+TP_2) while the lowest growth was recorded in the control (2.60e) Table 1.

Cob length and Diameter: The Analysis of variance showed that, the effect of *Azotobacter, PSB* and interaction between them on cob length and diameters were significant. The comparison of the mean values of the cob length and diameter for interaction between different biofertilizers showed that combine treatment of *Azotobacter* and *PSB* (TA+TP_1), (TA+TP_2), (TA+TP_3) had the highest length and diameter as compared to control in variety Eco 92 Table 1.



CONCLUSION:

It is concluded that, the treatment of biofertilizer increase the morphological and yield parameters more effectively than the control. The use of biofertilizer influenced the Maize variety Eco-92 positively. The application of biofertilizers as a source in agricultural production, and its proper use is an environmentally friendly way of strengthening plant growth and improvement for farmers.

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PHYSICOCHEMICAL PROPERTIES OF NATURAL POLYMER USED AS NANOCOATING OF FRUITS

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ABSTRACT: *The Inflorescence of Parkia biglandulosa, flowers of Bombax ceiba, Spathodia campanulata, fruits of Muntingia calabura, Artocarpus heterophyllus, Bridelia scandens leaves of Jaquinia armilaris were used for the isolation and characterization of natural Polymers. The thin film of natural polymer was prepared with different ingredients along with Corn Zein and applied for the coating of Tomato and Grape fruits. The physiocochemical properties of natural polymer were analysed. It was noticed that the tapped density (0.90 gm/cc) of Parkia biglandulosa and Muntingia calabura shows higher than the other mucilage powder, while the bulk density of Parkia biglandulosa and Muntingia calabura was (0.66 gm/cc). The Carr's Index and Hausner's ratio of Artocarpous (fruit) was (92.3 %) higher than the other mucilage. The oil absorption of Muntingia calabura and Spathodea canpanulata was higher than the other mucilaginous powders. The Water holding capacity of Muntingia calabura mucilage was 6 to 7 times higher than the other mucilage. The zeta potential of the mucilage powder of Parkia biglandulosa, Bombax ceiba, Muntingia calabura and Spathodea campanulata was higher than other mucilages which improves the flow properties of natural polymers. These natural polymers with better viscosity is helpful to move the various drugs or nutrients through chewing gum, butter, chocolate, margarin candy as well as other food items, this will improves the quality of these products. The results of the present study indicates that the mucilage isolated from various plants indicates the tapped and bulk density in the range of 0.60 to 0.90 gm/cc which indicates the tendency of mucilage particle to adhere to one another. Hence due to slimy solution of mucilage in water and its insolublity in organic solvents, it can perform as best medium in disintigrant and binder or it might be utilized as a gelling agent due to its sticky nature in various pharmaceutical formulations as well as it might be utilized as a best coater for various types of fleshy fruits during its post harvest storage.*

Keywords: *Physicochemical properties, Corn Zein, Zeta potential.*

INTRODUCTION :

Nature has provided us a wide variety of materials to help improve and sustain the health of all living things either directly or indirectly. Mucilage is a plant based polymers studied for their significant role in pharmaceutical dosages, it act as film coating agent, emulsifiers, bioadhesives and binders.The mucilage are characterized for physiocohemical powder characteristic, these are semi-synthetic and synthetic expients, due to lack of toxicity low cost, availability, soothing action and non-irritant nature. (Dwgade et. al 2012). Gums and mucilages are typically heterogeneous polyuronides with similar composition which upon hydrolysis, they yield sugars such as arabinose, galactose, glucose, mannose, xylose and various uronic acids (Kokate et al, 2002)

Gums and mucilages are widely used natural materials for conventional and novel dosage forms, (Umeshkumaret al, 2012). The synthetic polymers have certain disadvantages such as high cost, toxicity, environmental pollution during synthesis, non-renewable sources, side effects, less patient compliance, etc (Young and Lovell 2014). While the advantages of natural plant based materials include low cost, natural origin, free from side effects, bioacceptable, renewable source, environmental-friendly processing, local availability (especially in developing countries), better patient tolerance as well as public acceptance, from edible sources, etc (Williams and Wilkins)

The need to explore more natural sources of gums in addition to those already known is becoming more demanding because of its wider application in pharmacy, food supplements, printing and binding industries. The primary motivation for their utilization in the foodstuffs, environmental, cosmetic and construction industries (Garcia et al, 2012). The inflorescence of *Parkia biglandulosa* Whight & Arn, flowers of *Bombax ceiba* Linn, *Spathodia campanulata* P.Beauv and fruits of *Muntingia calabura.*

MATERIALS AND METHODS :

Young mature inflorescence of Parkiya biglandulosa, fruits Bridelia scandens, Muntingia calabura , flowers of Bombax ceiba, Spathodia campanulata and leaves of Jaquilina armilaris were collected form adjoining hills of Panhala Dist Kolhapur, and Shivaji University campus, Kolhapur.

Extraction of Mucilage

The mucilage content was extracted first 100 g of plant part that is mature fruits, leaves and flowers of each plant were extracted in 1000 ml of distilled water and boiled for 15 min. It was filtered through buckner funnel, without filter paper. Residue again boiled for 15 min and filtered through musclin cloth. Then equal volume of ethanol was added to precipitate the mucilage. Precipitate was condensed by using rotary evaporator. Precipiated mucilage was dried in oven 40^{o} c. The yield of mucilage was stored in airtight container under room temperature and used for further analysis.

Tapped density (M. R. Shivalingam 2010).

Ten gram of plant powder taken in 100 ml measuring cylinder and tapped for 1000 times and record tapped volume of the powder.

M = mass of the powder, Vt = tapped volume of the powder.

Tapped density (Dt) = M/Vt in (gm/ wt)

Bulk Density (M. R. Shivalingam 2010).

Ten gram of plant powder poured in 100 ml measuring cylinder and record the volume of powder in measuring cylinder.

M = mass of the powder, Vo = Bulk volume of the powder

Bulk density (Db) = M/Vo in (gm/ wt)

Oil Absorption capacity (Raghavendra et al 2007)

Take 0.25 g of plant powder then add ml of oil take weight and record oil absorbed sample weight.

Oil absorption = oil absorbed sample weight - Dry sample weight / Dry sample weight. (g oil/ g dry sample wt).

Water holding capacity (Raghavendra et al 2007)

Take 0.125 g dry mucilagenous plant powder add 12.5 ml of distilled water then record wet sample weight.

Water holding capacity = wet sample wt - dry sample wt / dry sample wt. in g powder / g dry sample wt.

Percentage compressibility or Carr's index (Rao et al 2014)

Carr's index (%) = tapped density - bulk density / tapped density X 100

Housner's Ratio (Rao et al 2014)

Hausner's ratio was calculated by a formula

Housner's Ratio **=** Tapped density / Bulk density

RESULT AND DISCUSSION:

The bulk and tapped density of mucilage obtained from different plant parts is shown in fig. No 1 and 2. The tapped density of *Parkia biglandulosa* and *Muntingia calabura* is higher than the other mucilage powder that is (0.90 gm/cc). While the bulk density of *Parkia biglandulosa* and *Muntingia calabura* is found to be 0.66 gm/cc.

The Carr's Index of different plant parts is shown in fig. No3. It is evident from the fig. that the Carr's Index of Artocarpous (fruit) is (92.3 %) higher than the other mucilage plant part such as inflorescence of *Parkia biglandulosa*, fruits of *Muntingia calabura, Bridelia scndens,* flowers of *Spathodea campanulata, Bombax ceiba* and leaves of *Jaquinia armilaris.*

The Hausner's ratio is the ratio of Tapped to the bulk density is shown in fig. no. 4. It is noticed that the ratio of *Artocarpous heterophyllous* and *Aegle marmelos* is higher than the other mucilage powders.

The Oil absorption capacity of mucilage obtained from various plant parts is shown in fig. no.5. It is noticed that the oil absorption of *Muntingia calabura* and *Spathodea canpanulata* is higher than the other mucilaginous powders.

The swelling index of mucilage powder obtained from various plant parts is shown in fig. no 6. It is noticed that the Water holding capacity of *Muntingia calabura* mucilage is 6 to 7 times higher than the other mucilaginous plant parts.

The tapped density (0.90 gm/cc) of *Parkia biglandulosa* and *Muntingia calabura* was higher than the other mucilage powder, while the bulk density of *Parkia biglandulosa* and *Muntingia calabura* was 0.66 gm/cc. The Carr's Index of *Artocarpous* (fruit) was (92.3 %) higher than the other mucilages such as inflorescence of *Parkia biglandulosa*, fruits of *Muntingia calabura, Bridelia scndens, flowers of Spathodea campanulata, Bombax ceiba and leaves of Jaquinia armilaris.* The Hausner's ratio of *Artocarpous heterophyllous* and *Aegle marmelos* was higher than the other mucilage powders. The oil absorption of *Muntingia calabura* and *Spathodea canpanulata* was higher than the other mucilaginous powders. The Water holding capacity of *Muntingia calabura* mucilage was 6 to 7 times higher than the other mucilage.

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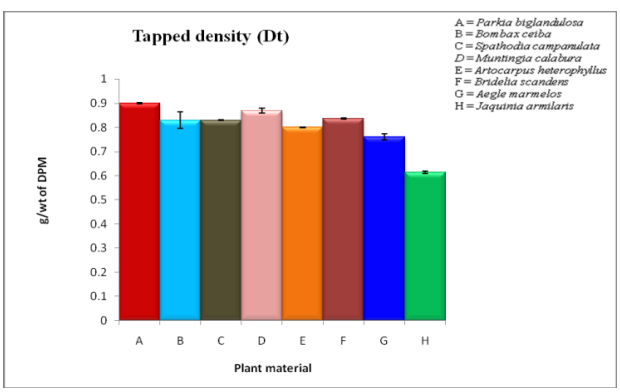


Fig. No. 1 Tapped density of Mucilagenous plants.

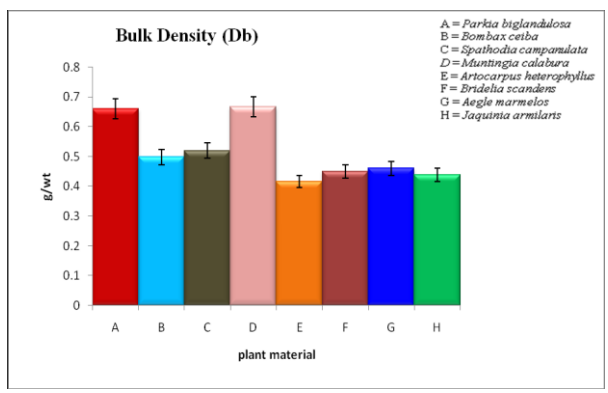


Fig. No. 2 Bulk density of Mucilagenous plants

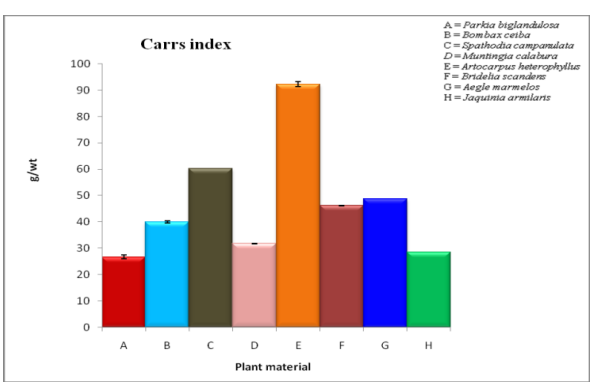


Fig. No. 3 Carr's Index of Mucilagenous plants.

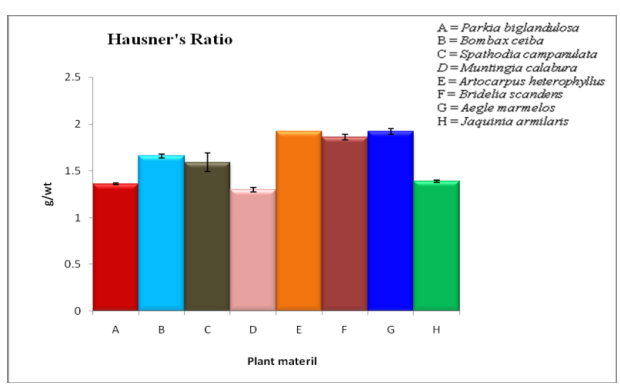
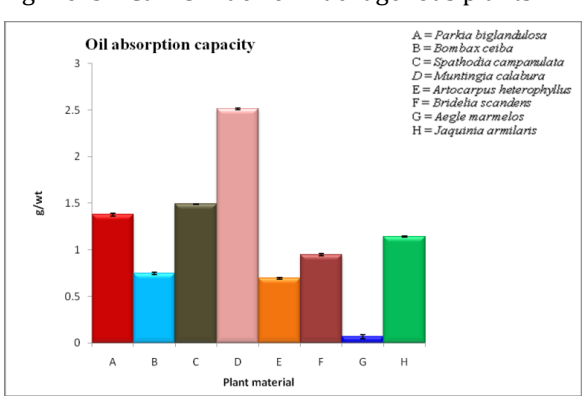


Fig. No. 4 Hausner's Ratio of Mucilagenous plants.



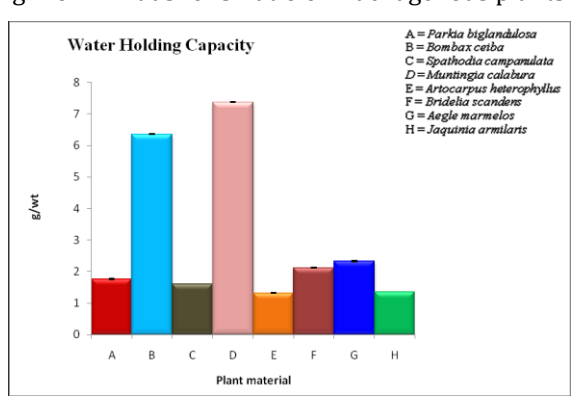


Fig: 5 Oil absorption capacity of Mucilagenous plants. Fig. 6 Water Holding Capacity of Mucilagenous plants.

EFFECT OF PASSAGE ON THE DEVELOPMENT OF CARBENDAZIM RESISTANCE IN *CURVULARIA PALLESCENS* CAUSING ROOT ROT OF *CORIANDRUM SATIVUM*

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ABSTRACT: *TheCoriandrum sativum(coriander) infected by Curvularia pallescens was observed as severe, in all pathogenscausing root rot disease.The MIC of carbendazim was carried out among abbreviated 15 isolates of Curvularia pallescens on Agar plate(in vitro) and root rot of coriander(invivo) exhibit variation i.e. from 300to3000µg/ml .In vitro and in vivo culturing of sensitive isolate of Curvularia pallescens on carbendazim, continuously, alternately and in mixture treatment showed varied results.*

Keywords: *Coriandrum sativum,Passage,Carbendazim, Curvularia pallescens.*

INTRODUCTION:

Coriandrum sativum L. is cultivated in Indian states like Maharashtra, Rajasthan, Gujarat, Madhya Pradesh,Tamilnadu, U.P; *etc*. It is green herb with strong smell which givearoma so used as cooking. The plant is infected by *Curvularia pallescens* and causes root rot disease to coriander (Dwivedi, *et al.*,1982).The valuable biochemical loss was seen after infection, on coriander.Survey of pathogen infecting coriander from 15 different localities of south west Maharashtra was carried out followed by MIC (Minimumu inhibitory concentration) using systematic fungicide carbendazim (Bavistin). Carbendazim is recommended for the management of coriander disease hence this fungicide was undertaken for detailed study. Report on fungicide root rot resistance crop plant pathogen of various crop plant are very few. Day by day there is increasing use of fungicideand resistance. The aim of the present investigation was therefore to examine the possibility of development of resistance and its management during passage in (a gap between two fungicide replication) *Curvularia pallescens*against carbendazim.

MATERIAL AND METHODS :

1.1 Materials

Infected sample of *Coriandrum sativum* causing root rot disease to coriander by *Curvularia pallescens* , fungicide like Bavistin (carbandazium 50% w/w), Roko (Thiophanate methyl 70% w/w), Sulfex (sulfer 80% w/w), Blue copper (copper oxide), kavach (Chlorothalonil 96% w/w Alkylnapthalene Sodium sulfonate 2.5% w/w , sodium lignosulphanite 2% w/w, calcium silicate 3% w/w), Indofil Z-78 (zineb - 75%w/w)Bavistin (Carbandazium 50% w/w), Roko (Thiophanate methyl 70% w/w), Sulfex (sulfer 80% w/w), Blue copper(copper oxide), kavach (Chlorothalonil 96% w/w Alkylnapthalene sodium sulfonate 2.5% w/w , Sodium lignosulphanite 2% w/w, Calcium silicate 3% w/w), Indofil Z-78 (Zineb -75%w/w)

1.2 Methods

The select fungicide Bavistin (carbandazium 50% w/w) was used for calculating MIC (Khandare and Kamble, 2013), along with other fungicides *in vitro* and *in vivo against Curvularia pallescens* Boedijn.

1.3 In*vitro* studies

Highly resistant isolate (Cp-8) in each passage was cultured on agar plates containing a sub lethal dose of carbendazim (3000µg/ml) i.e. 3%. The plate without fungicide served as control. Inoculum from the colony of previous passage, of the same isolate was placed at the center of each plate. In each passage linear growth was measured after 12 days. The percentage in increase of growth of isolate from passage to passage was considered as increase in carbendazim resistance. The development of resistant was studied up to fourth passage. Continuous, as well as alternate and mixed passage of carbendazim (Bavistin) with Roko, Sulfex, Blue copper, Kavach and Indofil Z-78. The concentration of fungicide was kept constant for all four passage.

1.4 *Invivo* studied:

In *vivo* studied roots of coriander were dipped in the solution of carbendazim (continuous), alternate and in mixture with select fungicides. They were inoculated with the wild sensitive isolate (Cp-9). An infected portion of the root of first passage was used as the source of inoculums for second passage. It

was continuously carried out up to the last passage. After 12 days of inoculums, the percentage of infection was recorded after incubation periods by 0-4 scale.

1.5 Statistical Analysis :

Statistical analysis was calculated using two way analysis of variance by Annova.

RESULTS :

***In vitro* studies:-**The result in vitro were obtained as follows The concentrations of fungicides were kept constant for all four passages.

Continuous and alternate treatment of carbendazim with select fungicides (*in vitro*)

Wild sensitive isolate (Cp-9) was cultured on plates containing carbendazim at MIC level 3% for four successive passages. It was seen that growing of *Curvularia pallescens*on the medium containing carbendazim for four successive passages continuously, significantly increased the resistance. When it was cultured alternately with other fungicides there was decrease in (radial growth) the development of carbendazim resistance than continuous exposure to fungicides. Roko, blue copper and indofil Z-78 show more inhibition of pathogen than alternate use of sulfex and kavach. Carbendazim when used alternately with roko, kavach, blue copper , sulfex and Indofil Z-78 there was complete inhibition of *Curvularia pallescens*at thirdpassage. The experiment showed that alternate use of all select fungicides reduced radial growth of pathogen than its exposure using continuous use of fungicide. (**Table 1**).

Table 1.Effect of exposure of *Curvularia pallescens*to carbendazim continuous and alternating with other fungicides on the development of carbendazim resistance during four successive passages (*in vitro*).

Fungicide	Passage no.1	Passage no.2	Passage no.3	Passage no.4
Carbedazim (Bavistin)individual	24.00	32.00	40.00	46.00
Carbedazim alternate Roko	36.00	26.00	00.00	00.00
Carbedazim alternate Sulfex	40.00	32.00	00.00	00.00
Carbedazim alternate Blue copper	39.00	25.00	00.00	00.00
Carbedazim alternate Indofil Z-78	32.00	26.00	00.00	00.00
Carbedazim alternate Kavach	38.00	29.00	00.00	00.00

P≤ 0.001

Continuous and mixed treatment of carbendazim with select fungicides (*in vitro*) :-

The important observation was that, there was complete inhibition of pathogen by fungicide roko, blue copper and indofil Z-78 at second passage. The application of fungicides in mixture was noticed to be more beneficial than its continuous and alternate *in vitro* use. Results are shown in (**Table 2**).

Table.2.Effect of exposure of *Curvularia pallescens* to the mixture of Carbendazim with other fungicides on the development of resistance during four successive passages (*in vitro*)

Fungicide	Passage no.1	Passage no.2	Passage no.3	Passage no.4
Carbedazim (Bavistin) individual	22.00	32.00	40.00	48.00
Carbedazim +Roko	26.00	00.00	00.00	00.00
Carbedazim + Sulfex	40.00	32.00	00.00	00.00
Carbedazim + Blue copper	28.00	00.00	00.00	00.00
Carbedazim + Indofil Z-78	29.00	00.00	00.00	00.00
Carbedazim + Kavach	38.00	23.00	00.00	00.00

P≤ 0.001

***In vivo* studies:-**

The results obtained were as follows

Continuous and alternate treatment of carbendazim with select fungicides (*in vivo*):-

It was observed that continuous treatment of carbendazim for four successive passages increased the resistance of pathogen. The percentage of infection was 25% (grade-1) at first and second passage and 50% (grade-2) at third and fourth passage.

When select fungicides were applied alternately with carbendazim, there was complete inhibition of the pathogen at first passage except sulfex. Alternate treatment of carbendazim with sulfex showed 25% infection (grade-1). During experiments, roko, kavach, blue copper and Indofil Z-78 showed complete

inhibition of pathogen at first passage. **(Table 3).**

Table.3. Effect of exposure of *Curvularia pallescens* to Carbendazim continuous and alternating with other fungicides on the development of resistance during four successive passages (*in vivo*).

Fungicide	% of infection and Grade	Passage number			
		1	2	3	4
Carbendazim (Bavistin) Individual	% of infection	25.00	25.00	50.00	50.00
	Grade	1	1	2	2
Carbendazim Alt. Roko	% of infection	00.00	00.00	00.00	00.00
	Grade	0	0	0	0
Carbendazim Alt Sulfex	% of infection	25.00	00.00	00.00	00.00
	Grade	1	0	0	0
Carbendazim Alt Blue copper	% of infection	00.00	00.00	00.00	00.00
	Grade	0	0	0	0
Carbendazim Alt Indofil Z-78	% of infection	00.00	00.00	00.00	00.00
	Grade	0	0	0	0
Carbendazim Alt Kavach	% of infection	25.00	00.00	00.00	00.00
	Grade	1	0	0	0

P≤ 0.001

Continuous and mixed treatment of carbendazim with select fungicides (*in vivo*):-

It was observed that continuous treatment of carbendazim for four successive passages increased the resistance of pathogen. The percentage of infection was 25% (grade-1) at first and second passage which was similar result like that was observed in alternate treatment. During experiment with use of mixed treatment of fungicides it was observed that all select fungicides except sulfex were able to inhibit complete growth of *Curvularia pallescens*. In sulfex there was noticed 25% (grade-1). The other select fungicides namely, roko, kavach, blue copper and Indofil Z-78 showed complete inhibition of *Curvularia pallescens.***(Table 4).**

Table.4.Effect of exposure of *Curvularia pallescens*to the mixture of Carbendazim with other fungicides on the development of resistance during four successive passages (*in vivo*)

Fungicide	% of infection and Grade	Passage number			
		1	2	3	4
Carbendazim(Bavistin) Individual	% of infection	25.00	25.00	00.00	00.00
	Grade	1	1	0	0
Carbendazim ₊Roko	% of infection	00.00	00.00	00.00	00.00
	Grade	0	0	0	0
Carbendazim + Sulfex	% of infection	25.00	00.00	00.00	00.00
	Grade	1	0	0	0
Carbendazim +Blue copper	% of infection	00.00	00.00	00.00	00.00
	Grade	0	0	0	0
Carbendazim +Indofil Z-78	% of infection	00.00	00.00	00.00	00.00
	Grade	0	0	0	0
Carbendazim + Kavach	% of infection	00.00	00.00	00.00	00.00
	Grade	0	0	0	0

P≤ 0.001

DISCUSSION :

In the present investigation, effect of passage was studied *in vitro and in vivo* using carbendazim and otherfungicide. During experiment it was observed that culturing *Curvularia pallescens* on the agar medium (*in vitro*) containing carbendazim for four successive passages continuously showed increased resistance against carbendazim in the pathogen (Table-1). This result was on same line as mentioned in where carbendazim resistance in the pathogens including *Curvularia pallescens* has been observed all over

the world. According to the pathologists working on this line are of the opinion that there was builds up of the resistance in the pathogen under various selection pressures under field conditions.

During study of four passages, effect of treatment of carbendazim alternately (*in vitro*) with *Curvularia pallescens* showed that there was total inhibition of infection at third passage when there was application of Roko, Kavach, Blue copper, Sulfex and Indofil Z-78.The fungicides when applied in mixture of carbendazim (*in vitro*) it was noted that Roko, Blue copper and Indofil Z-78 completely inhibited growth of pathogen at second passage. But it was also observed that during application of these fungicides alternately (*in vivo*) roko, kavach, blue copper and Indofil Z-78 showed complete inhibition of pathogen at first passage except sulfex. The mixed treatment showed that there was complete inhibition of pathogen by roko, kavach, blue copper and Indofil Z-78. The important observation during experiment was that, the use of carbendazim in mixture was very beneficial for cultivar when applied on *Coriandrum sativum* (*in vivo*) and the continuous treatment of carbendazim increased resistance at every passage. Similar observation was noted by (Khandare, 2014) where Horsten, (1979), Hartill (1983), Kable and Jaffery,(1980); (Kamble, 1991), Mikaberidze (2015), Bosch, *et.al*., (2014), Hobbelen *et al*., (2013), Hollomon , (2015) Ishii, (2015), (Fuchs *et al;* 1977; Brown and Hall, 1979; Dekker and Gielink, 1979a and b). .

CONCLUSION :

During study of four passages, effect of continuous treatment of carbendazim increased resistance at every passage. Thealternate (*in vitro*) application with *Curvularia pallescens* showed that there was total inhibition of infection at third passage but when fungicides applied in mixture of carbendazim (*in vitro*) it showed complete inhibition of pathogen at second passage. So application of fungicide in mixture was observed to be more beneficial than alternate use. Similar results were obtained in vivo.The important observation during experiment was that, the use of carbendazim in mixture was very beneficial for cultivar when applied on *Coriandrum sativum*.

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Lecideopsella arnaudii (C. and M. Moreau) Dopare and Patil *Comb. Nova.* A Black Mildew from Western Ghats -I

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ABSTRACT: *In present paper a species of Lecideopsella from Loculoascomycetes is described as comb. nova. viz. Lecideopsella arnaudii (C. and M. Moreau), Dopare and Patil comb. nova. The species is reported on new host.*

Keywords: *Black mildew, Fungi, Lecideopsella, Loculoascomycetes, Taxonomy.*

INTRODUCTION :

Genus *Lecideopsella* Hohnel. Was established by Hohnel (1909), However genera like *Gymnopeltis* Stev. and *Plectomyriangium* Moreau were merged into genus *Leptophyma* Sacc. by Arx and Muller (1975) in their re-evaluation of bitunicate ascomycetes. But Eriksson and Hawksworth (1987) treated *Leotophyma* Sacc. invalid and not classified in their classification due to its uncertain affinity and kept into a valid genus *Lecideopsella* Hohnel.

Plectomyriangium arnaudii (C. and M. Moreau) was reported on *Lophira alata*. This genus is merged in *Lecideopsella* by Hohnel (1909) The present collection matches with taxonomical description (as reported by C. and M. Morreau, on *Lophira alata*) as *Plectomyriangium arnaudii.* Therefore, the genus of present collection is treated as *Lecidceopsella* and species is described as *Lecideopsella arnaudii*(C. and M. Moreau)as combination nova.

MATERIAL AND METHODS :

The diseased plant material was collected from Amboli forest and deposited in paper envelopes after the host identification. Specimen was examined by usual laboratory methods by staining with cotton blue, melzers reagent etc. The specimen identified by referring recent and most upto date Literature. The specimen was deposited in the department herbarium of college as well as in the H.C.I.O.New Delhi.

RESULT AND DISCUSSION :

Lecideopsella arnaudii (C. and M. Moreau) Dopare and Patil combination nova.
(Syn=*Plectomyriangium arnaudii* C. and M. Moreau), Rev. Mycologia, Paris, N.S. 24 : 348-355,1959)

Free mycelium absent, ascomata hypophyllous, 290-540 µm in diameter; asci globose to ovoid or spherical, sessile, 8-spored, 37-54 × 41-50 µm ascospores elliptical,1-septate, hyline, smooth, cells 2-3 guttulate, 20-29×7-9µm., conidial state absent.

Holotype- On living leaves of *Diospyros candolleana* wight. (Ebenaceae), Amboli, M. S. Leg. S.R.Yadav, Isotype- H.C.I.O. 41010.

Table 1: Comparative account of *Lecideopsella* and present collection.

	Ascomata µm in Diameter	Asci µm	Ascospores µm	Host
Present Collection	Hypophyllous 290 × 540	37-54 ×41-50	20-29×7-9	*Diospyros candolleana*
(Original) Syn= *Plectomyriangium arnaudii* C. and M. Moreau.	350	40-50	23-27 ×9-11	*Lophira alata*

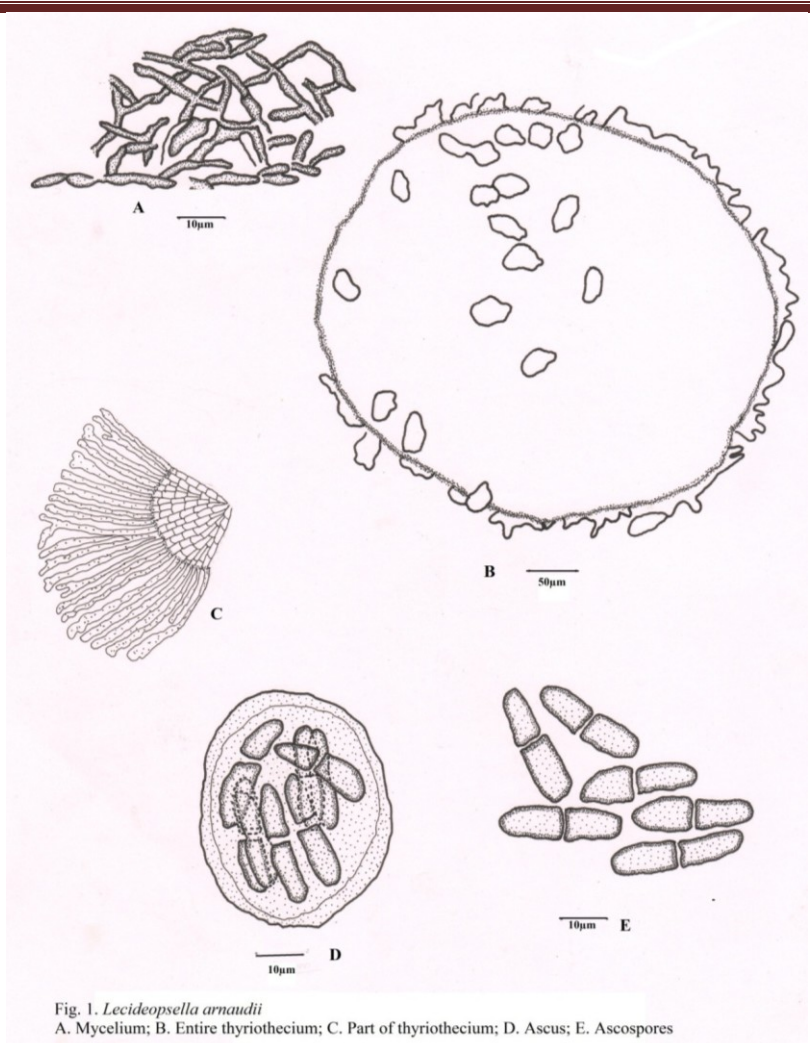

Fig. 1. *Lecideopsella arnaudii*
A. Mycelium; B. Entire thyriothecium; C. Part of thyriothecium; D. Ascus; E. Ascospores

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EFFECT OF FERRIC REDUCING ANTIOXIDANT PLASMA ACTIVITY ON TWO VARIETIES OF GRAPES

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ABSTRACT: *Grape is one of the most commercial horticultural crops of the world. Grapes are very nutritious, rich source of minerals and different vitamins. It revels from the figure that the FRAP activity of Sonaka seedless treated with + sulphur and Thompson seedless treated with K_2CO_3+ sulphur + coating, is greater than the other raisins. It is also observed that the all the treatment shows quit significant higher FRAP activity in both seedless varieties.*

Keywords: *FRAP, Thompson seedless, Sonaka seedless.*

INTRODUCTION :

Grape (Vitis sp.) belonging to Family Vitaceae is a commercially important fruit crop of India. Grapes are eaten as raw or they can be used for making wine, raisins, jam, and jelly, which are very nutritious and rich source of minerals like potassium, phosphorus, calcium, magnesium and other micronutrients and different vitamins. The dried grapes, commonly known as raisins, have a great importance in economy of the country and considered as one of the nutritious most popular dry fruits in the world. Raisins are dried fruits of certain varieties of grapevines with a high content of sugar and solid flash (Khair and Shah, 2005). The increased production of table grapes has a great potential to produce raisins with minimum losses of fresh fruits (Telis et al., 2004.According to De Candolle (1886), the cultivation of grape goes back to 4000 BC in Egypt and the oldest wine was found in Armenia near the Caspian Sea in Russia. As per the report of Parker et al. (2007), the Thompson seedless grapes, were first introduced in 1876, accounted for 95% of the California crop used for golden raisin production. Thapar (1960) indicated that grape was introduced in India in 1300AD by the Persian invaders in North and South India (Daulatabad in Aurangabad districts of Maharashtra). Nizam of Hyderabad has also introduced some grape varieties into Hyderabad from Persia in the early 20th century (Chadha and Shikhamany, 1999). India is a small producer of grapes, with a world share of less than 2 percent (Barrientos and Kritzinger, 2004). The total average cultivation of grape is near about 80,000 hectares in India and 28,000 hectares in Maharashtra.

MATERIAL AND METHODS :

The ferric reducing / antioxidant power (FRAP) assay was used to measure the total antioxidant power of raisin. In the FRAP assay, reductants (antioxidants) in the sample reduce Fe3+/tripyridyltriazine complex, present in stoichiometric excess, to the blue colored ferrous form, with an increase in absorbance at 593 nm. Antioxidant activity assays were performed by the method described by Benzie and strain, (1996). The results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

RESULT AND DISCUSSION :

The ability of raisins to chelate iron (II) is presented in Figure . The Thompson seedless raisins treated with K2CO3+sulphur+coating+ Orange essence are capable of high chelating iron (II) than the thopmson seedless raisins treated with other treatments. Sonaka seedless treated with +sulphur shows higher chelating activity than of the other Sonaka seedless raisins. One of the mechanisms of antioxidative action is chelation of transition metals, thus preventing catalysis of hydroperoxide decomposition and Fenton-type reactions (Gordon, 1990). In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. According to Aboul-Enein et al. (2003), the transition metal ion, Fe2+ possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals. The greater ferrous ion chelating activity in methanolic extract of Crataegus pentagyna and Sambucus ebulus was determined by Ebrahimzadeh et al., (2008). Ndhlala et al., (2006) noticed that velvet sweet-berry has highest reducing powers as compared to red ivory which are higher

than in the previously recorded in jackal berry by them. Al- Muwaly et al., (2012) reported 81% of inhibition in black grapes.

The extract obtained from Sonaka seedless raisins treated with K2CO3+sulphur and Thompson seedless raisins treated with K2CO3 + sulphur+ coating+ orange essence showed the most active extract interfered with the formation of ferrous and ferrozine complex, suggesting that it has greater chelating activity and captures ferrous ion before ferrozine than the other treatments. Transition metal ions, catalyses the initiation and decomposition of hydroperoxides, contribute to lipid oxidation which is the main source of degradation of food products (Antolovich et al., 2002). The higher levels of ferrous ion chelating ability of the K2CO3+sulphur treated raisins will certainly improve the antioxidant potential of raisins and also helps to protect the membranes of raisins from lipid peroxidation. Which will helps to improve the texture of raisins. Hence, the metal chelating activity of raisins can be of potential interest in the food industry.

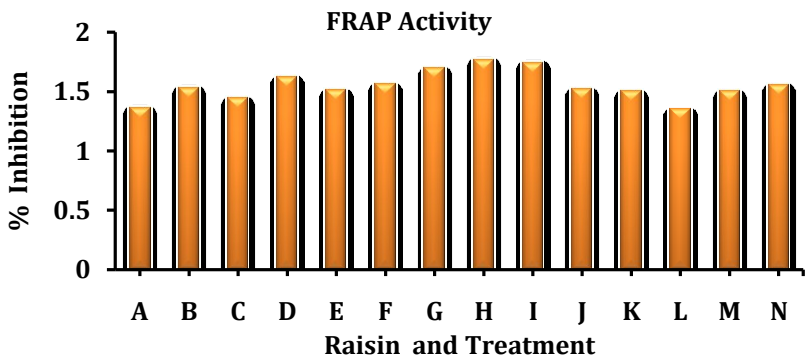

Fig. 1: Changes in FRAP activity of treated raisin varieties.

A = Thompson seedless treated with MgCO3, B= Sonaka seedless treated with MgCO3, C=Thompson seedless treated with K2CO3, D=Sonaka seedless treated with K2CO3, E=Thompson seedless treated with CaCO3, F=Sonaka seedless treated with CaCO3, G=Thompson seedless treated with K2CO3+ sulphur, H= Sonaka seedless treated with K2CO3+ sulphur, I=Thompson seedless treated with K2CO3+ sulphur + coating, J=Sonaka seedless treated with K2CO3+ sulphur +coating, K=Thompson seedless treated with K2CO3+ sulphur + coating + Mango essence, L=Sonaka seedless treated with K2CO3+ sulphur +coating + Mango essence, M=Thompson seedless treated with K2CO3+ sulphur + coating + Orange essence, N=Sonaka seedless treated with K2CO3+ sulphur +coating + Orange essence

SUMMARY AND CONCLUSION :

The FRAP activity of Sonaka seedless treated with K2CO3+ sulphur and Thompson seedless treated with K2CO3+ sulphur + coating, showed greater activity than the other raisins, suggesting that treatment are more suitable and may store the polyphenols from the natural degeneration have very high primary antioxidant activity. FRAP assay suggests that the both the raisins exhibits greater reducing ability. The availability of polyphenols in the raisins of both the species seems to be an important factor dictating the antioxidant and free-radical- scavenging capacity of fruits. The antioxidant efficiency determined by the present FRAP assay depends on the redox potentials of the Phenolic compounds present in the fruits, characterized by the complexity of their molecules. From our findings, it is apparent that the reducing ability of polyphenols, as determined by the FRAP assay, seems to depend on the degree of hydroxylation and extent of conjugation of the phenolic compounds available in the raisins.

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ACETYL SALICYLIC ACID MEDIATED ION ACCUMULATION IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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ABSTRACT: *Groundnut is the major annual oilseed crop of India. It requires all macronutrients and micronutrients for its growth and development, relatively in higher quantity as compared to other plants. Groundnut is susceptible to nutritional disorders. Thus the present investigation has been carried out on the influence of various concentrations (5, 50, 100 and 200 ppm) of ASA on ion accumulation in groundnut leaves and roots. All the applied foliar treatments of ASA showed stimulatory effects on the accumulation of micronutrients and macronutrients. The significant accumulation of ions was observed with higher concentration of ASA (100 ppm). Present study signifies that ASA contribute to enhance productivity potential of groundnut through mineral nutrition.*

Keywords: *Acetyl salicylic acid, groundnut, ion accumulation, minerals.*

INTRODUCTION:

India is the world's largest producer of oilseed legume crop groundnut. Seeds of groundnut are rich source of proteins, lipids, fatty acids, minerals and vitamins. Groundnuts are also a good source of calcium, phosphorus, iron, zinc and boron. Groundnut crop requires all the micro and macronutrient elements for its growth and development. The peanut nutrition is inimitable as the pod develops under soil and seed nutrition is occur through pod relatively those transported from root, shoot and back to seed (Singh, 1999). Several workers reported nutrient requirements of groundnut crop (Dwivedi, 1988, Singh, 1999). The groundnut crop is susceptible to nutritional disorders due to insufficient supply of nutrients (Beringer and Taha, 1976). Nutritional disorders causes reduction in yield of groundnut.

Thus it is essential to develop practices which can reduce nutrition depletion and induce crop production. In this view the effect of Acetyl salicylic acid (ASA) on ion uptake in groundnut was studied. Acetyl salicylic acid also termed as 'Aspirin' is acetyl derivative of salicylic acid. Earlier studies emphasized ameliorated effect of ASA on total nitrogen, oil and protein content, fatty acid contents and PR-proteins of groundnut (Jadhav and Bhamburdekar, 2015; Jadhav, 2014, 2018).

MATERIALS AND METHODS:

The seeds of groundnut cultivar SB-11 were procured from agricultural research station, Karad. The experiment was carried out in randomized complete block design with three replications. The 30-days old plants were sprayed with different concentrations of (5, 50, 100 and 200 ppm) ASA. The seedlings receiving foliar sprays of distilled water served as control. The leaf and root samples were collected from each treated set, washed thoroughly with distilled water and oven dried at 60^{o}C. An acid digest was analyzed for inorganic constituents. Ca and K concentrations were analyzed with flame photometer (Jenway PEP7 Felsed, Dunmow, Essex, UK), Zn was determined by using autoanalyzer, phosphorus by colorimetric method, Mg, Fe and Mn were determined by atomic absorption spectrophotometer.

RESULTS AND DISCUSSION:

The influence of foliar application of ASA on ion accumulation is depicted in Fig. 1 to 10. The results showed that all the applied concentrations of ASA significantly enhanced nutrient contents of leaves and roots. The profound uptake of ions was recorded with the higher concentration of ASA. The significant content of molybdenum was found in roots than in leaves. According to Singh (1996) application of phosphorus enhances nitrogen metabolism and nitrogen content of kernel and foliage. It shows close conformity with our findings that ASA mediated increased content of phosphorus definitely improved nitrogen fixation (Jadhav, 2018). Potassium content in leaves of groundnut was found to be effectively increased by the treatment of higher concentration of ASA i.e. 100 and 200 ppm whereas in roots noteworthy levels of potassium was observed with 50 and 100 ppm ASA treatments. These findings show close resemblance with the reports of Kang *et al*. (2010). The stimulated potassium levels will prove

beneficial in increasing activities of antioxidant enzymes, photosynthetic pigments and productivity of groundnut.

The increased accumulation of nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, zinc, molybdenum, boron and sulphur in leaves and roots of groundnut in response to ASA will definitely induce nitrogen fixing capacity of root nodules, leghemoglobin content of nodules, disease resistance, shelling percentage, fatty acid content by mitigating nutrient deficiencies. Earlier studies reported ASA mediated increased protein content, oil content, increased yield, total nitrogen and soluble nitrogen fractions and PR-proteins in groundnut (Jadhav and Bhamburdekar, 2014, 2015; Jadhav, 2018), it might be due to stimulated nutrient contents. Thus our results indicate that ASA contribute to enhance productivity potential of groundnut through mineral nutrition.

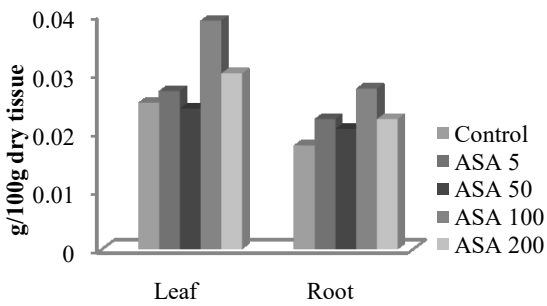

Fig. 1 Effect of ASA on Phosphorus…

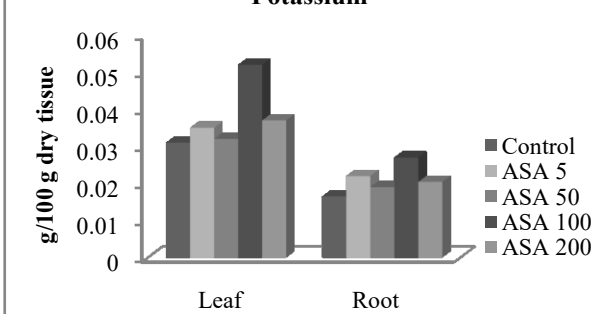

Fig. 2 Effect of ASA on Potassium Content

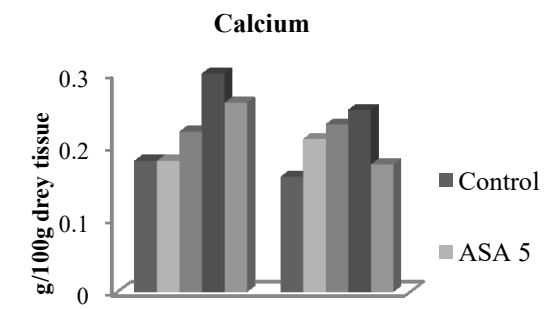

Fig. 3 Effect of ASA on Calcium content

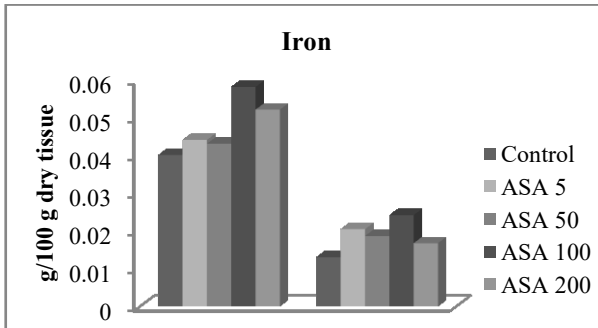

Fig. 4 Effect of ASA on Iron Content

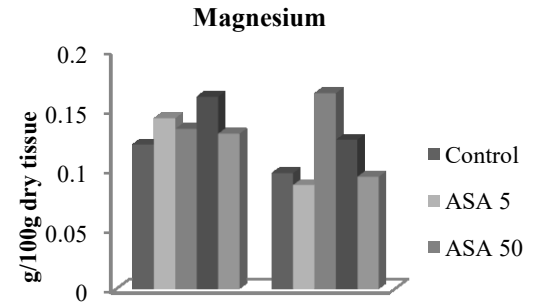

Fig. 5 Effect of ASA on Magnesium Content

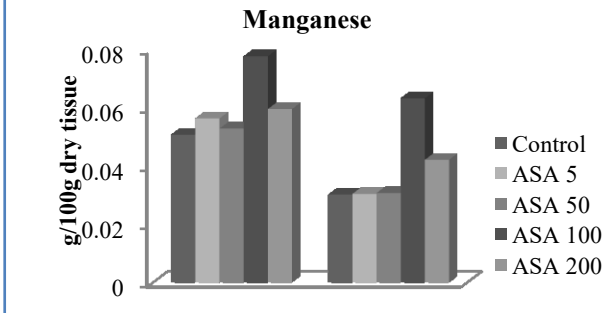

Fig. 6 Effect of ASA on Manganese Content

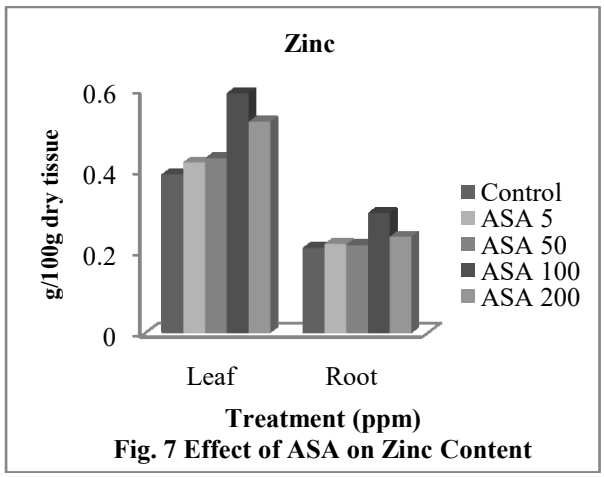

Fig. 7 Effect of ASA on Zinc Content

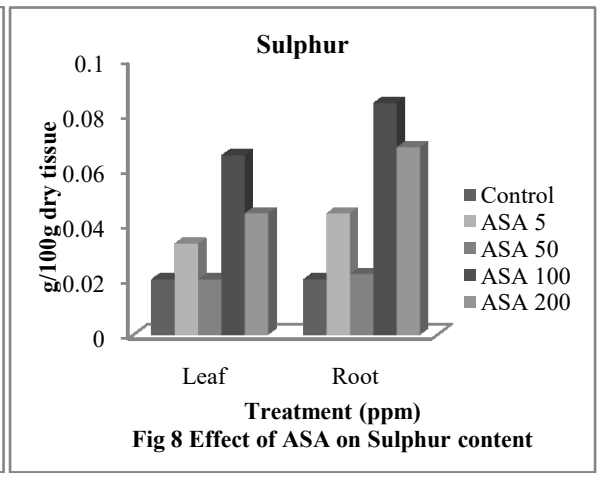

Fig 8 Effect of ASA on Sulphur content

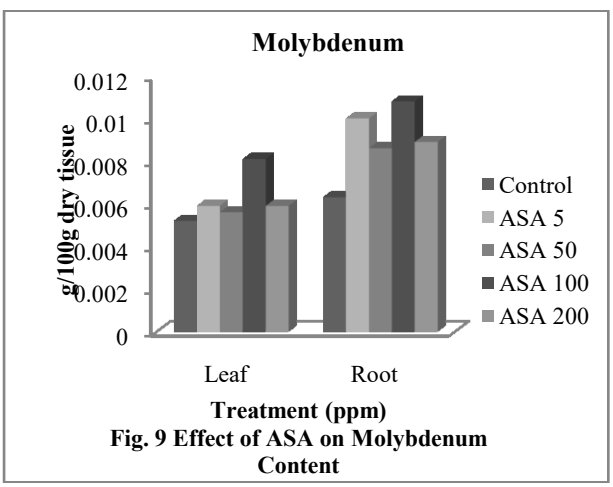

Fig. 9 Effect of ASA on Molybdenum Content

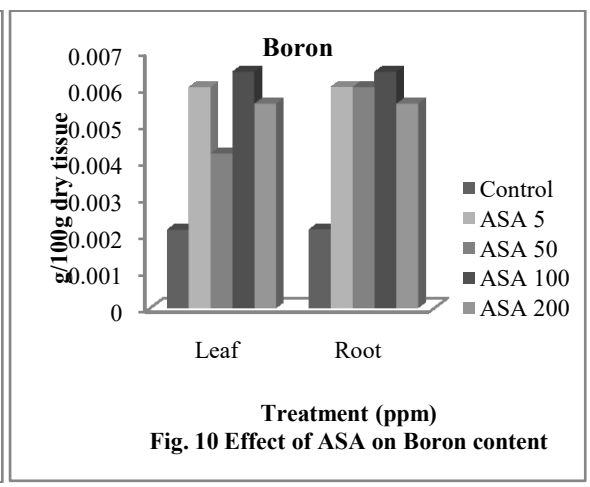

Fig. 10 Effect of ASA on Boron content

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EVALUATION OF *TRICHODERMA SPP.* FOR BIOCONTROL OF CHARCOAL ROT OF MAIZE

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Kolhapur. 416004 (M. S) India.

ABSTRACT: *Trichoderma spp. are fungi that occur worldwide. Recent studies show that they are not only parasites of fungal plant pathogens but also can produce antibiotics. In addition, certain strains can induce systemic and localized resistance to several plant pathogens. Moreover, some strains may enhance plant growth and development. The potential of Trichoderma species used as biological agents of plant diseases have been known since the 1930s.There was variation in MICs of carbendazim among the sixteen isolates of MacrophominaphaseolinaTassi (Goid) causing charcoal rot of Zea mays L. Var. African tall in vitro tested by food poisoning technique. Among these sixteen isolates, isolate Mp3 was sensitive tolerating 1 ppm carbendazim while Mp9 was resistant and its MIC was 450 ppm. So, to manage such carbendazim resistance in M.phaseolina six Trichoderma spp. were screened against carbendazim sensitive and resistant isolates of MacrophominaphaseolinaTassi (Goid) causing charcoal rot of maize by dual culture technique for their biocontrol potential. Among the six spp. of Trichoderma,T. pseudokoningii showed maximum antigonastic potential (79.63%) against resistant isolate of Macrophominaphaseolina . In remaining species of Trichoderma, T. koningiopsis showed 78.40% inhibition, T. virens showed 76.33%, T. atroviride showed 73.71%, T. viride showed 69.63%, and T. harzianum gave 67.04% inhibition of Macrophominaphaseolina. Similarly T.koningiopsis gave maximum inhibition of carbendazim sensitive isolate of Macrophominaphaseolina (82.96%) followed by T.pseudokoningii (80.74%) ,T.virens (79.63%), T. atroviride (77.41), T. viride (75.93) and T. harzianum(69.63%).*

Keywords: *Trichoderma spp.MacrophominaphaseolinaTassi (Goid), fungal plant pathogens, resistance*

INTRODUCTION :

Members of the genus*Trichoderma* are known as Zygomycotinous fungi, fast growing in culture and produce numerous green asexual spores. These occur worldwide and are commonly associated with root, soil and plant debris (Howell *et al.*, 2003). These have long been recognized as biological agents to control plant diseases. Since the first application in 1930s, *Trichoderma* species became popular biological agents to protect crops against plant pathogens all over the world.

More recent research indicated that certain strains of *Trichoderma* can induce systemic and localized resistance to several plant pathogens. Plants treated with *Trichoderma* in the root zone can produce higher levels of peroxidase, chitinase activity, deposition of callose-enriched wall appositions on the inner surface of cell walls and pathogenesis-related proteins. Moreover, some strains may enhance plant growth and development. These phenomena was observed by several researchers who treated plants with *T. harzianum* resulting in large increases in root area and cumulative root length, as well as significant increase in dry weight, shoot length, and leaf area over that of the untreated control (Howell, 2003).

Due to effective control of plant diseases, several commercial biological products based on *Trichoderma* species are manufactured and marketed in Asia, Europe and USA for use on a wide range of crops. These can be efficiently used as conidia, mycelium and chlamydospores which are produced in either solid state or liquid fermentation (Harman *et al.*, 2004). The use of *Trichoderma* as a biological agent of plant diseases has long been known, however, these were introduced in Vietnam only in the last two decades (Tran, 1998). Research has been done on biological control potential of *Trichodermaspp*. against several pathogens attacking vegetables, fruits, field and industrial crops. Experiment conducted in present work showed that inhibition of pathogens (both sensitive and resistant to carbendazim) by different *Trichodermaspp.* (Table 1).

MATERIAL AND METHODS :

To study biocontrol of charcoal rot of maize different *Trichoderma* spp. were selected.The experiments were carried out by as per technique used by Morton and Stroube(1955). In present work antagonistic acivity of *Trichodermavirens, T, atroviride, T. viride, T. harzianum, T.koningiopsis,* and

T.pseudokoningii were examined by using dual culture technique on CDA medium. 20 ml CDA medium was poured in to sterilized petriplates. 6 mm disc of actively growing mycelium was aseptically picked from 5 day old culture of *Trichoderma spp.* and *Macrophominaphaseolina* were placed on opposite side of the plate (equal distance from periphery). Triplicates of both sensitive Mp-3 and resistant Mp-9 isolates were maintained and incubated at 30 ± 2^0 C. For control plates at the opposite side of sensitive and resistant isolates of *Macrophominaphaseolina* 6 mm sterile agar disc were placed and radial mycelial growth was measured at specific time intervals up to 5 days. In each treatment inhibition of radial mycelial growth were recorded and calculated by using formula (Lokesh and Benagi, 2006).

Inhibition percent of mycelial growth = (C-T/C) X 100 Whereas,

C = Radial mycelial growth in mm of *Macrophominaphaseolina* in control plates.

T = Radial mycelial growth in mm of *Macrophominaphaseolina* in treated plates.

Mycelial fragments from interaction region between *Macrophominaphaseolina* and *Trichoderma*spp. were picked with the help of sterile forcep, stained with cotton blue and observed under microscope.

Table.1 . Antagonistic activity of *Trichoderma* spp. against sensitive and resistant isolates of *Macrophominaphaseolina* by using dual culture technique.

Sr. No.	Antagonist	% inhibition of mycelial growth	
		Sensitive Mp3	Resistant Mp9
1	*Trichodermavirens*	79.63	76.33
2	*Trichodermaatroviride*	77.41	73.71
3	*Trichodermaviride*	75.93	69.63
4	*Trichodermaharzianum*	69.63	67.04
5	*Trichodermakoningiopsis*	82.96	78.4
6	*Trichodermapseudokoningii*	80.74	79.63

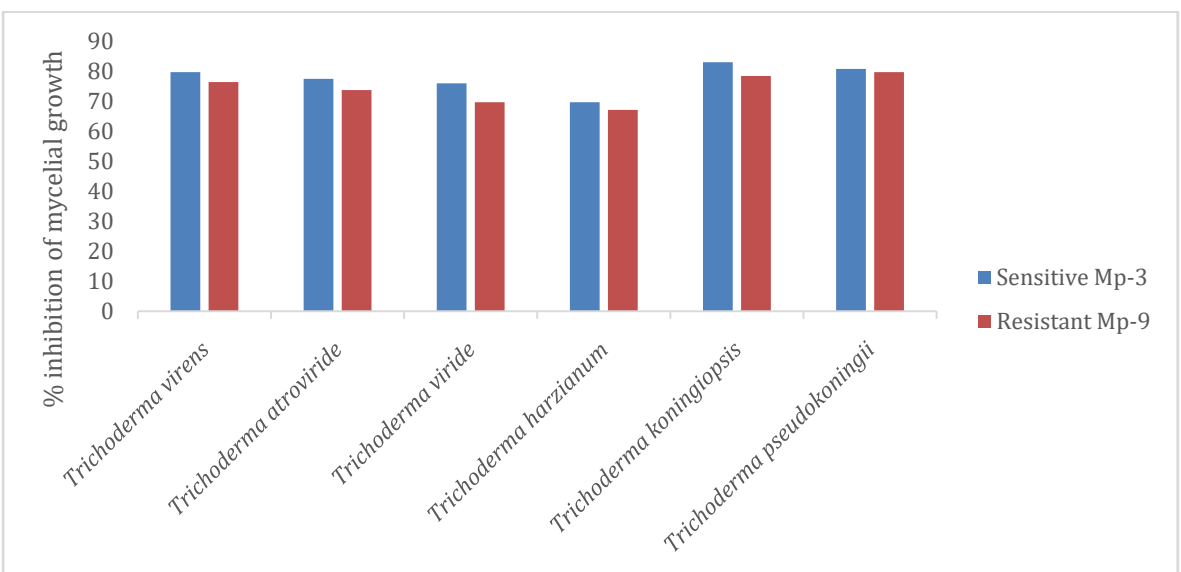
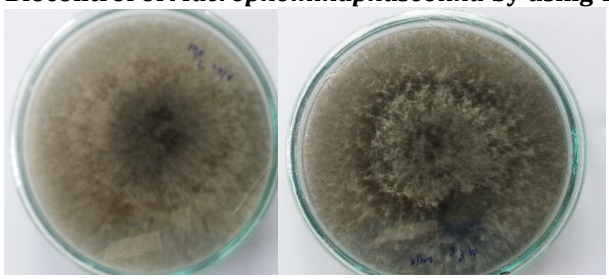
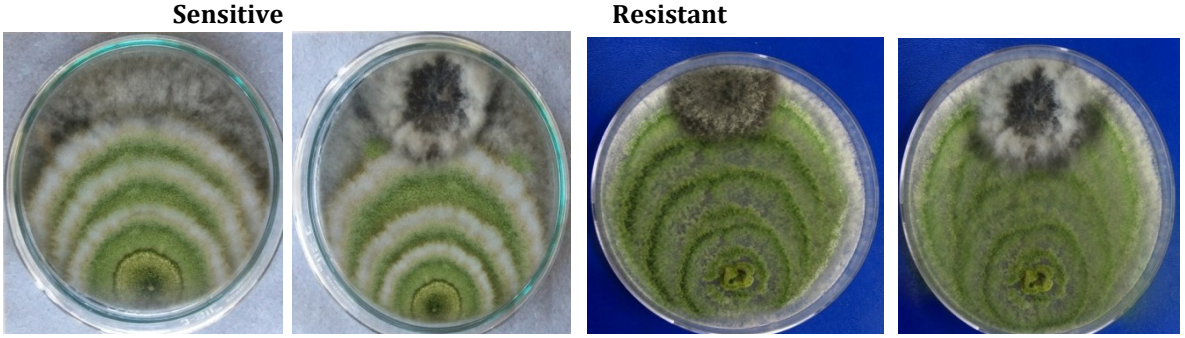


Fig.1. *In vitro* inhibition of growth of *Macrophominaphaseolina* by *Trichodermaspp*. in dual culture method

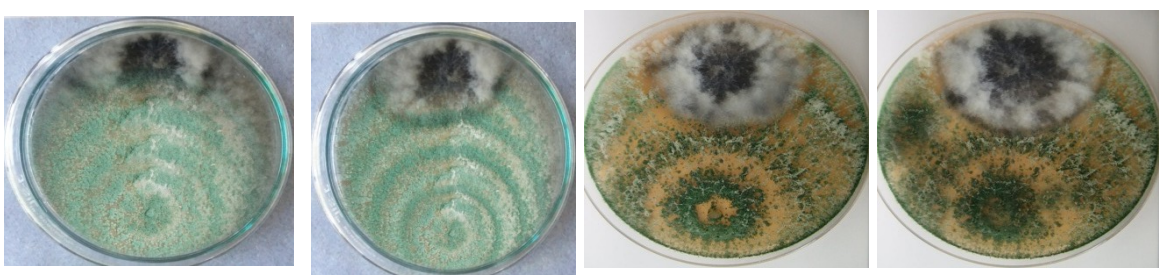
Biocontrol of *Macrophominaphaseolina* by using *Trichodermaspp*.



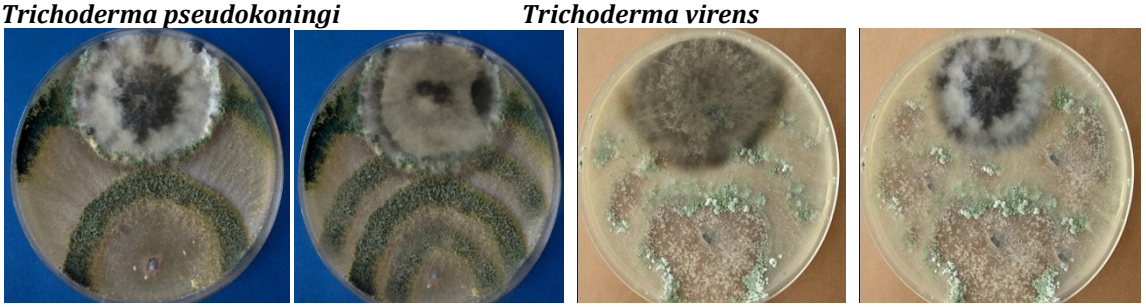
Sensitive **Resistant**



TrichodermakoningiopsisTrichoderma viride



Trichoderma pseudokoningi ***Trichoderma virens***



Trichoderma harzianumTrichoderma atroviride

RESULTS AND DISCUSSION :

The advantages of use of biocontrol includes ecofriendly, cost effective and best results. It is clear from the results of this investigation that *Trichodermaspp*. has very great potential of antagonistic activity against *Macrophominaphaseolina*. *Trichodermakoningiopsis* was gave maximum inhibition of both sensitive and resistant isolates of *Macrophominaphaseolina*

The results strongly indicatemycoparasitism in *Trichoderma spp*. Coil formation is a common symptom of mycoparasitism and leads to the death of pathogen. *Trichodermaspp*. have very great potential of antagonistic activity against *Macrophominaphaseolina*. *Trichodermaviride* and *T..harzianum*were comparatively effective in controlling the growth of sensitive and resistant isolates of *Macrophominaphaseolina*.The growth inhibition was the maximum in the case of *Trichodermaviride*followed by *T.harzianum*and *T.hamatum.*

In vitro antagonism byvarious antagonistic fungi on pathogenic organisms is a field of study in

which reports are constantly thronging. In the light of recent emphasis on biological control, this area is gaining special attention. The pre requisite for any attempt to suggest a suitable biocontrol agent against a particular is to screen with all the available antagonists. The possible mechanisms proposed to explain the antagonism are competition. Antibiosis, lysis and hyperparasitism are the events in biological control (Park, 1960).Antagonistic action of*Trichoderma spp.* studied by several workers ((Dhingra and Sinclair, 1977; Wyllie, 1988: Olaya andAbawi, 1996)).The inhibitory action of culture filtrates of *Trichoderma spp.* might be due to their production of inhibitory volatile metabolite (Dennis and Webster, 1971). (Ghisalberti andSivasithamparam, 1991) gave a detailed account about the antifungal antibiotics produced by *Trichoderma spp.* The lysis and growth inhibition of the pathogen involvesexcretion of lytic extra cellular β-1,3glucanases and chitinases by *Trichoderma spp.* in the growth medium and soil. This causes lysis of the cell wall of the pathogen, which might also be a reason for the growth inhibition in the presence of antagonists (Chet and Baker, 1981).

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EFFECT OF CULTURE MEDIA ON COLONY CHARACTER AND FUNGAL DIVERSITY OF RHIZOSPHERE SOIL SAMPLES FROM BARAMATI OF PUNE DISTRICT (MS) INDIA

Vilas V. Kamble[1] & Chandrakant J. Khilare[2]
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ABSTRACT: *Various fungi isolated from rhizosphere soils of Cynodon dactylon L. and Cyperus rotundus L. were tested on different culture media, their levels of PH and sporulation were highly affected by culture media. Differences in growth of fungal mycelium and their sporulation were observed on two culture media from the rhizosphere soil of Baramati. Microscopic observations revealed that, significant increase in Penicillium species and Aspergillus niger on Potato Dextrose Agar, while Chaetomium funicola and Fusarium roseum showed highest growth on Czapek's Dox Agar. Deuteromycetes were isolated in large numbers in these species. The results obtained on PDA and CDA were used for analysis during Kharif and Rabi seasons. The fungal species diversity in the rhizosphere depends upon seasons and culture medium. Results suggest that Kharif season harbor diverse communities.*

Keywords: *Fungal diversity, Baramati, Kharif season, Rabi season, Deuteromycetes*

INTRODUCTION:

Fungal growth in an artificial culture medium is influenced by several chemical and physical parameters. There is vital role of culture medium on microbial diversity (Tortora and Funk, 1995). Fungal growth observed in variety of natural habitats and they require many specific elements during their life cycle phases (Singh and Sharma, 2014) . Wide range of culture media are available for isolation of fungi in laboratory that influence growth, morphology and pigmentation which depends upon culture medium, light, temperature, P^H, available water and atmospheric gas mixture in adjacent area (Kumara and Rawak, 2008). Best mycelium growth and sporulations of *Alternaria alternate* was obtained in PDA followed by Czapek's Dox Agar medium (Rajashree R. Pawar et al., 2014).

Review of literature indicated that some plant species have been extensively studied for research of fungi in the rhizosphere (Mandeel, Q. A. 2002). Several fungi have been isolated from non-crop plants (Abdel-Hafez, 1982; Al- NurEl- Amin and A.M.A. Shadabi, 2007) However scanty reports are available of distribution of rhizosphere soil fungi in grass species. (Gomes et al. 2003; Hyakumachi et al., 1992; Porras Alfaro et al., 2008; Smith et al., 2008). The variation in fungi from rhizosphere differ depending upon locality, season and type of the soil (Berg et al., 2005). Fungi are identified on the basis of morphological characters. Previous studies on grass rhizosphere soil fungi indicated large number of fungal species of which very few were isolated and used for their biocontrol and resistance induce ability (Dewab et al., 1989,Domesch et al., 1980, Meera et al., 1994, Narita and Suzui, 1991; Orole and Adejumo 2009). These studies are useful for documentation of fungal diversity in rhizosphere soils of grasses. On the basis of this survey of rhizosphere soil fungi in *Cynodon dactylon* L. and *Cyperus rotundus* L.was undertaken which indicated the dominance of Deuteromycetes and some non sporulating fungi.

The present stdy aimed to understand the diversity of fungi in both Kharif and Rabi seasons in rhizosphere of *Cynodon dactylon* L. and *Cyperus rotundus* L.in the Baramati area; Maharashtra,India.

MATERIALS AND METHODS:

Baramati is located at 18.15^0 N and 74.58^0 E . This has variety of flora and fauna. This area has an average elevation of 538 meters and received annual rainfall of 502 mm. temperature ranging from 18 – 31^0 C. The study area us characterized by well drained black cotton soil. Experiments were conducted during Kharif and Rabi seasons of 2015-16 and repeated during 2016-17.

Selection of grass species:

Cynodon dactylon L. and *Cyperus rotundus* L.were identified based on standard manuals and the flora of the presidency of Bombay.

Collection and analysis of soil samples:

The grass species from study sites and labeled for collection of soil samples. The roots adjacent to roots of each plant from the surface was collected in replicates of three, in polythene bags. These soil samples were air dried and sieved and 100 g of the sample was used for physic-chemical properties.

Preparation of rhizosphere samples and isolation of fungi:

Six replicates of *Cynodon dactylon* L. and *Cyperus rotundus* L. were uprooted carefully from the soil and soil adhering roots were collected in polythene bags and taken to the laboratory. The root system of grasses was removed carefully by gentle spatula and brush. Samples were used for experiments within five hours.

Soil fungi were enumerated by serial soil dilution mehod (Waksman, 1994) on PDA and CDA media within 24 hours. Remaining soil samples were sieved through 2 mm mesh size to remove coarse material and used for physicochemical analysis.

The incubated petriplates for 120 lbs pressure for 20 minutes at 121^0C and allow to cool and kept at room temperature at 25^0C. Three replicates of plates were prepared for each sample. Fungi were mounted in lactophenol and stained with Cotton blue.

Rhizosphere samples were diluted ($10^{-4)}$ plating on PDA and CDA (Himedia laboratory Mumbai) amended by supplementation with streptomycin (100 mg /L) (Dhingra and Sinclair, 1993). The plates inoculated were kept in incubation chamber at 23^0 C for 4-6 days. The fungal species were identified on the basis of cultural characters, fruiting bodies andspores with the help of standard identification manuals (Barnett; 1960, Ellis; 1976; Nagmani and Manoharachary, 2006). The number of fungal colonies (cfu g^{-1} soil) produced on plates in each sample were determined.

Statistical analysis:

The replicate trials of two years were subjected to homogeneity of trials by tow way ANOVA.

RESULTS:

There was no significant (P 0.05) variation in the occurrence of fungi associated from both the grasses at Baramati. Hence the two years data was averaged season wise.

Fungal occurrence and types of media:

Fungal species were characterized on the basis of morphological characters. In comparison to other medium PDA exhibited large number of fungal isolates. Most of them were found common to both PDA and CDA. However, *Chaetomium funicola* Kunze ex Fr. and *Fusarium roseum* Link. were exclusively observed on CDA likewise *Aspergillus niger* Van Tieghem, *Penicillium oxalicum* Currie, J.N.; Thom, C. and *Mucor hiemalis* Wehmer were observed on PDA.

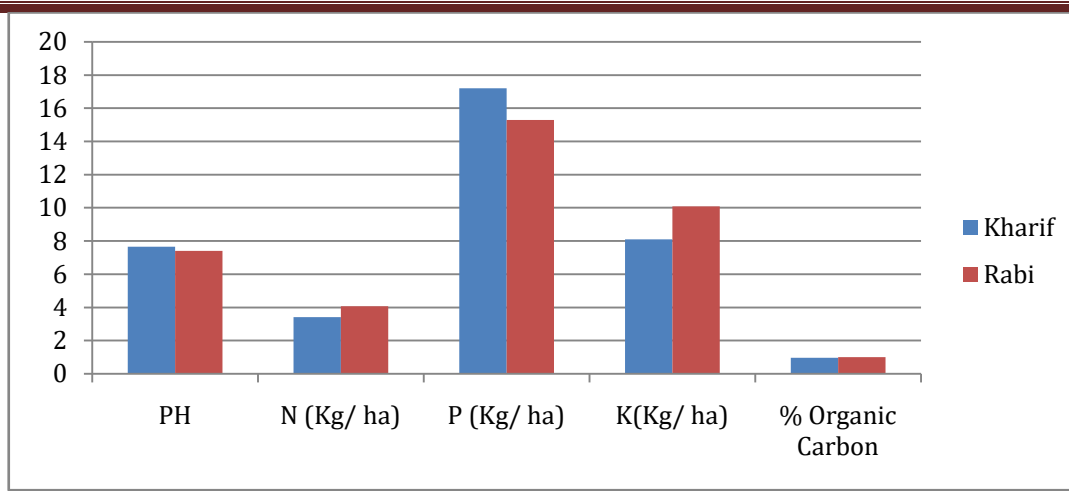
Soil character:

N,P,K and available percent organic carbon from soil samples differ in both Kharif and Rabi seasons but were almost similar during the respective seasons. (Table 1)

Table 1. Mineral nutrient composition of soil samples collected from rhizosphere soil at Baramati:

Season	PH	N (Kg/ ha)	P (Kg/ ha)	K(Kg/ ha)	% Organic Carbon
Kharif	7.65	3.42	17.20	8.10	0.97
Rabi	7.40	4.06	15.30	10.08	1.00

Note: Data based on two seasons of two trials.



Composition of media and fungal diversity:

Numerous fungal species were isolated from the rhizosphere samples. Most fungal isolates occurred on both PDA and CDA media however fungal incidence was more on PDA as compare to CDA medium. Some species such as *Rhizopus nigricans* Ehrenb, *Aspergillus candidus* Link ex. Fries , *Aspergillus clavatus* Desmazieres , *Aspergillus flavus* Link ex. Fries , *Aspergillus nidulans* (Eidam) Winter, *Aspergillus niger* Van Tieghem, *Aspergillus ochraceus* Wilhelm, *Aspergillus stellatus* Curzi, *Aspergillus tamari* Kita, *Aspergillus terreus* Thom and *Aspergillus versicolor* Vuillemin were recorded on PDA while others such as *Chaetomium funicola* Cooke, *Monilia sitophila* (Montagne) Saccardo , *Penicillium scleroteorum* J. L. Pitt. and *Fusarium roseum* Link. were recorded on CDA.

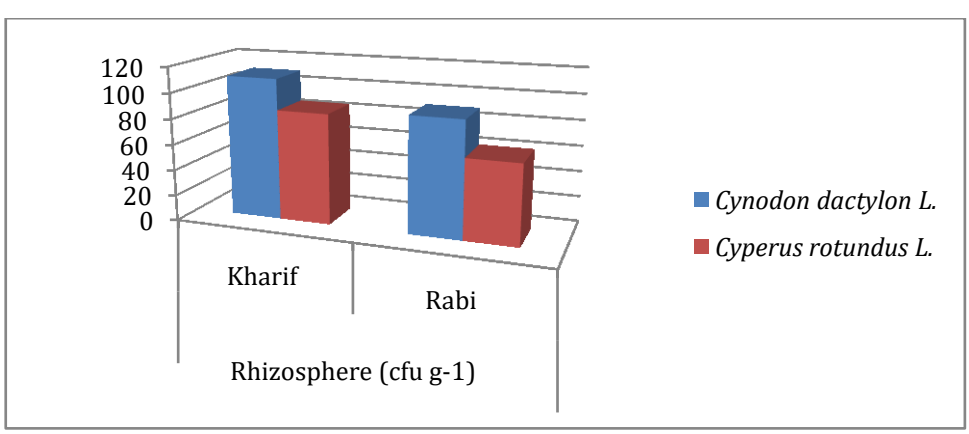
Distribution of fungi in rhizosphere:

The rhizosphere samples from both the grasses *Cynodon dactylon* L. and *Cyperus rotundus* L. yielded 192 fungal isolates per gram of sol on PDA. The number of fungal isolates in the rhizosphere varied depending on the soil sample and type of grass species. *Cynodon dactylon* L. exhibited higher fungal incidence (200 cfu g^{-1} soil) in its rhizosphere through all seasons whereas *Cyperus rotundus* L. showed 148 (200 cfu g^{-1} soil). Table 2.

Table 2. Occurrence of fungal species in the rhizosphere soil.

Grass	Rhizosphere (cfu g^{-1})	
	Kharif	**Rabi**
***Cynodon dactylon* L.**	110	90
***Cyperus rotundus* L.**	86	62

Note: Data based on four seasons of two trials.



In total 21 species from 10 genera were observed. The fungi could grouped into Phycomycetes, Ascomycetes and Deuteromycetes. Some of the prominent fungi from rhizosphere grasses included

Rhizopus, *Aspergillus*, *Alternaria*, *Cladospora, Fusarium*, *Penicillium*, and *Trichoderma*.

Species of *Chaetomium funicola* Cooke, *Penicillium scleroteorum* J. L. Pitt. and *Fusarium roseum* Link.were predominant on CDA while *Rhizopus nigricans* Ehrenb, *Aspergillus candidus* Link, *Aspergillus niger* Van Tieghem, *Aspergillus ochraceus* Wilhelm, *Aspergillus stellatus* Curzi, *Aspergillus tamari* Kita, *Aspergillus terreus* Thom and *Aspergillus versicolor* Vuillemin were prominent on PDA. Table 3.

Table 3. Frequency of rhizosphere soil fungi of grass species on PDA and CDA.

Sr. no.	Fungi	Percentage frequency occurrence			
		Cynodon dactylon L.		*Cyperus rotundus* L.	
	Ascomycetes	PDA	CDA	PDA	CDA
1	*Mucor hiemalis*	1	0	2	1
2	*Rhizopus nigricans*	2	4	4	4
3	*Rhizopus stolonifer*	3	3	4	3
	Ascomycetes				
4	*Chaetomium funicola*	0	12	0	6
	Deuteromycetes				
5	*Alternaria alternata*	8	3	6	2
6	*Alternaria brassicicola*	6	5	5	2
7	*Alternaria solani*	9	2	7	3
8	*Aspergillus candidus*	9	3	5	5
9	*Aspergillus clavatus*	2	1	2	4
10	*Aspergillus flavus*	5	4	4	
11	*Aspergillus nidulans*	4	7	8	
12	*Aspergillus niger*	24	22	25	21
13	*Aspergillus ochraceus*	1	9	4	2
14	*Aspergillus* species	1	1	2	2
15	*Aspergillus stellatus*	2	2	1	3
16	*Fusarium roseum*	2	8	3	21
17	*Memnoniella subsimplex*	8	3	4	4
18	*Monilia sitophila*	6	2	4	2
19	*Penicilliumoxalicum*	0	6	0	10
20	*Penicillium scleroteorum*	3	2	5	2
21	*Trichoderma viridae*	3	1	5	3

Note: Data is an average of three replicates each.

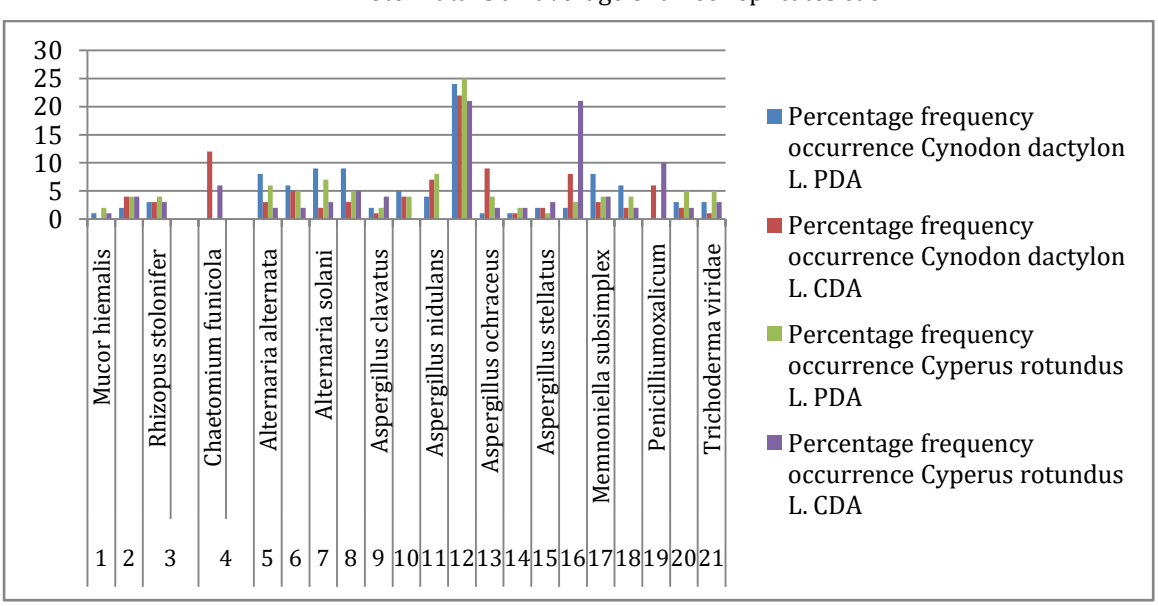
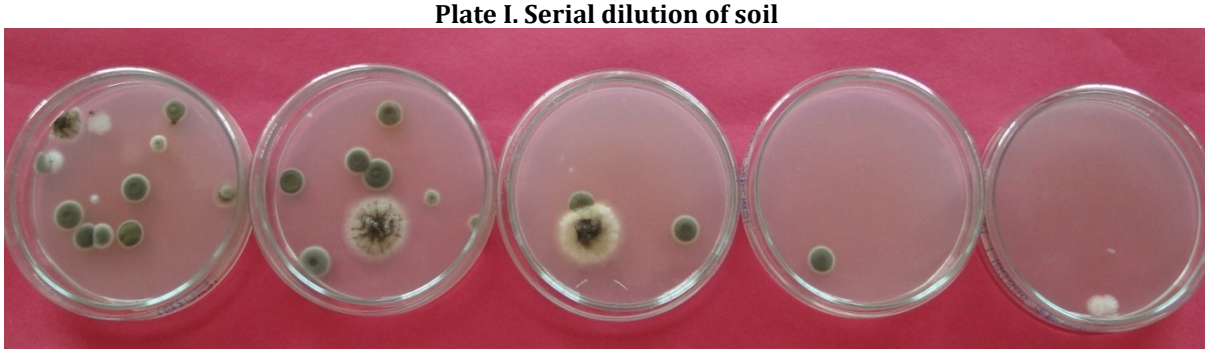
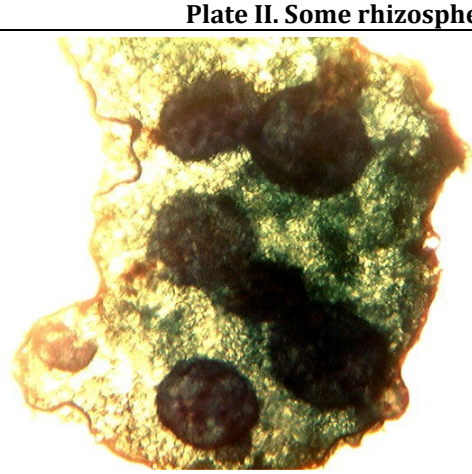


Plate I. Serial dilution of soil

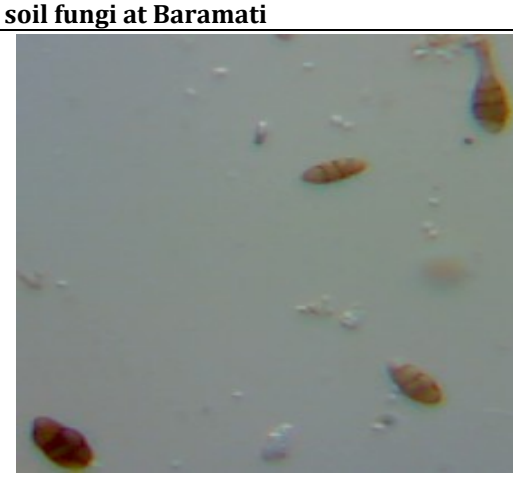


Pure cultures of soil fungi on culture media

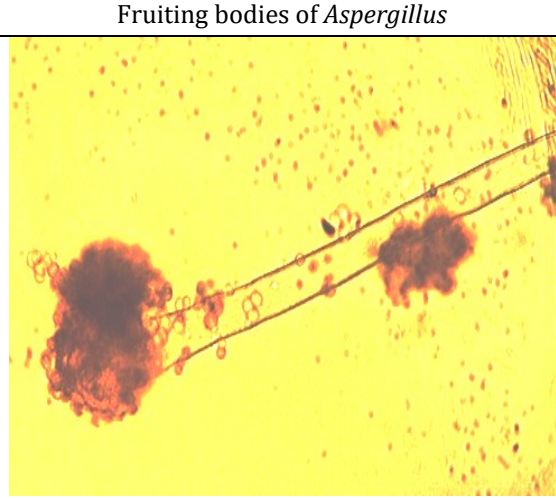
Plate II. Some rhizosphere soil fungi at Baramati



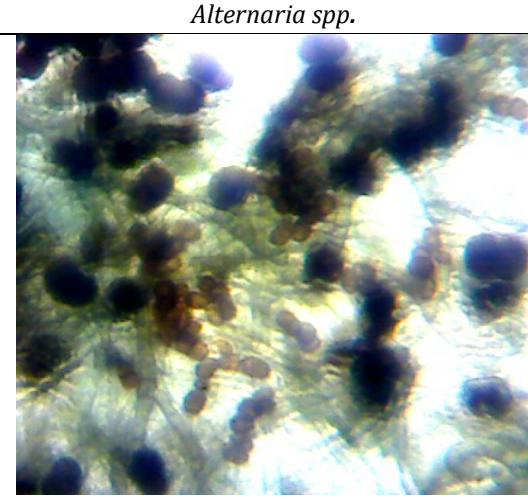
Fruiting bodies of *Aspergillus*



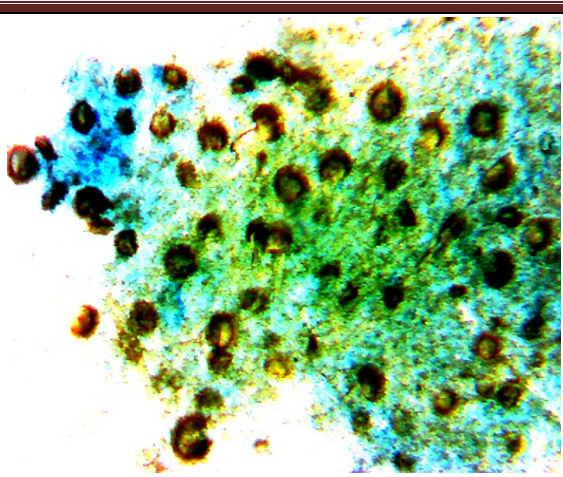
Alternaria spp.



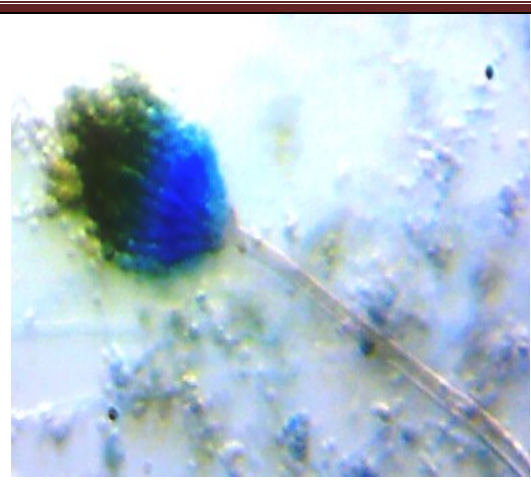
Aspergillus ochraceous



Cladosporium cladosporoides



Aspergillus species



Penicillium oxalicum

DISCUSSION:

Result of the study indicated that there is dominance of Deuteromycetes members in the studied samples. *Aspergillus* was found most dominant in both the seasons and both culture media. This may be due to adaptability of *Aspergillus* in most alkaline soils and rich mineral nutrients. The root exudes has vital role in the rhizosphere. This might be favorable for growth of *Aspergillus* and Deuteromycetes representatives.

Potato dextrose agar showed a large number of fungal species, suggests its effectiveness as a culture medium for culturable fungal study.

Cynodon dactylon L. and *Cyperus rotundus* L. harbor variety of fungi in both Kharif and Rabi seasons. *Cynodon dactylon* L. exhibited more qualitative and quantitative diversity as compare to that of *Cyperus rotundus* L. The fungal occurrence vary to some extent depends upon the season. Some of the fungi are potentially useful as biological control agents. Rhizosphere fungal communities vary temporally and hence there is scope for isolation of more number of fungi that could be exploited for their uses.

It is concluded from the findings that soil rich in organic carbon, holds better moisture and creates good mycoflora in the rhizosphere soils of grasses.

ACKNOWLEDGEMENTS:

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ADDITION TO THE MELIOLACEOUS FUNGI FROM WESTERN GHATS, INDIA

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ABSTRACT: *In present paper one variety and a species belongs to family Meliolaceae are taxonomically described as new to science. These are, Meliola roureae Sydow. var. minor var. nov. on Rourea minor and Asteridiella senegalensis sp. nov. on Maytenus senegalensis. A critical comparison was done for each taxon with earlier records and comparative account is provided here.*

Keywords: *Asteridiella – Bhimashankar Wildlife Sanctuary – Black mildew –Meliola – new taxa.*

INTRODUCTION :

Sahyadri ranges or Northen Western Ghats in India are noticeable with wide variation in rainfall patterns and the region's complex geography, hence it produces a great variety of vegetation types. Among these ranges, Bhimashankar Wildlife Sanctuary is situated on the crest of Thane and Pune districts (Rahangdale & Rahangdale 2017). As the part of Western Ghat, this region is blessed with abundant life forms and habitats, which favors the growth of black mildew fungi.

Black Mildews or Dark mildews are black colony forming fungi and they are very peculiar for their structures and habitat. They are obligate, superficial and believed to be host specific in nature. They infect mostly wild plants and are less destructive as well they do not show any pathogenic effect on host plant hence they received less attention of mycologists. The black colony forming parasitic fungi belong to several taxonomic groups, viz. Hyphomycetes, Meliolales, Schiffnerula and its anamorphic forms, Asterinales, Meliolinaceae, etc. (Hosagoudar 2009). The family Meliolaceae comparises 8 genera with 2385 species infecting 194 host families (Zeng *et al*. 2017).

During a study of black mildew fungi from Bhimashankar and its surrounding area, authors came across to, two undescribed black milews. The detail microscopic examination revealed that, it belongs to a species of genus *Asteridiella* and a variety of genus *Meliola*. The earlier records did not match with the morpho-taxonomical characters of both taxon therefore, present paper proposes new species of the genus *Asteridiella*, namely *Asteridiella senegalensis* sp.nov. and a new variety of *Meliola roureae* Sydow namely *M. roureae* var. *minor* var. nov.

MATERIAL AND METHODS :

The leaves and twigs of host plants, infected with black mildew fungi were observed in the field, field notes were made regarding date of collection, nature of colonies, and locality. The specimens were collected and brought to the laboratory in separate sterilized polythene bags from study area in winter season (2017-2018). Host plants were identified by using Flora of Maharashtra (Singh & Karthikeyan 2000). The specimens were pressed neatly and dried in blotting papers; these well dried specimens were kept in standard size herbarium packets. The fungal species were identified and their distributional records were checked by using relevant literature (Bhise 2015; Bilgrami et al.1991; Farr 2018; Hansford 1961; Hosagoudar 2008, 2009, 2013; Jamaluddin 2004; Zeng 2017). Type specimens were deposited at Herbarium Cryptogamae Indiae Orientalis (HCIO), IARI, New Delhi (India), for accession. For identification and taxonomical studies, both micro and macro-morphological characters were used. To observe the natural structure of colonies according to their mycelial branching, position of appresoria and phialides, structure of mycelial setae; a drop of peeling solution was applied on selected colonies as a thin layer (Patil & Patil, 2017), after drying the film was mounted in the same peeling solution (Xylene-Thermocol solution). For microscopic preparation, lactophenol and cotton blue were used and 20 observations are recorded. Morpho-taxonomical details were observed under compound light microscope and photomicrographs were taken under Leica DM 2000 fluroscence microscope equipped with digital camera; illustrations were prepared with Camera Lucida (mirror type).

RESULTS :

Taxonomy

1. Meliola roureae* var. *minor Lonkar, Patil & Salunkhe *var. nov.*

MycoBank MB 826828

Beeli Formula: 3111:4222

Etymology: The specific epithet is based on name of the host species.

Type: India, Maharashtra: Bhimashankar Wildlife Sanctuary, on living leaves of *Rourea minor* (Gaertn.) Alston (Connaraceae); 08/01/2018, HCIO 52169.

Colonies hypophyllous, subdense, circular to spreading, confluent, black, up to 4 mm in diameter. Hyphae undulate to crooked, branching opposite to irregular at wide angles, closely reticulate, cells 14-29 × 7-9 µm. Appresoria bicelled, alternate to unilateral, straight to bent, antrorse, subantrorse to spreading, 14-30 × 11-18 µm; stalk cell cylindrical, 3-11 × 7-11 µm; head cell obovate, clavate, oblong, straight, bent to geniculate, 11-21 × 11-18 µm. Phialides mixed with appresoria, ampulliform, opposite to alternate, 16-29 × 7-14 µm. Mycelial setae many, simple, straight, apex mostly acute, rarely dentate, scattered & grouped around perithecia, up to 404 µm. Perithecia globose, verrucose, closely scattered, up to 182 µm. Ascospores 4-septate, oblong, cylindrical, slightly constricted at septa, brown, 41-47 × 16-20 µm.

Habitat and Distribution: Inhabiting living leaves of *Rourea minor* (Gaertn.) Alston (Connaraceae), on the way to Gupt Bhimashankar, Bhimashankar Wildlife Sanctuary, Maharashtra, India.

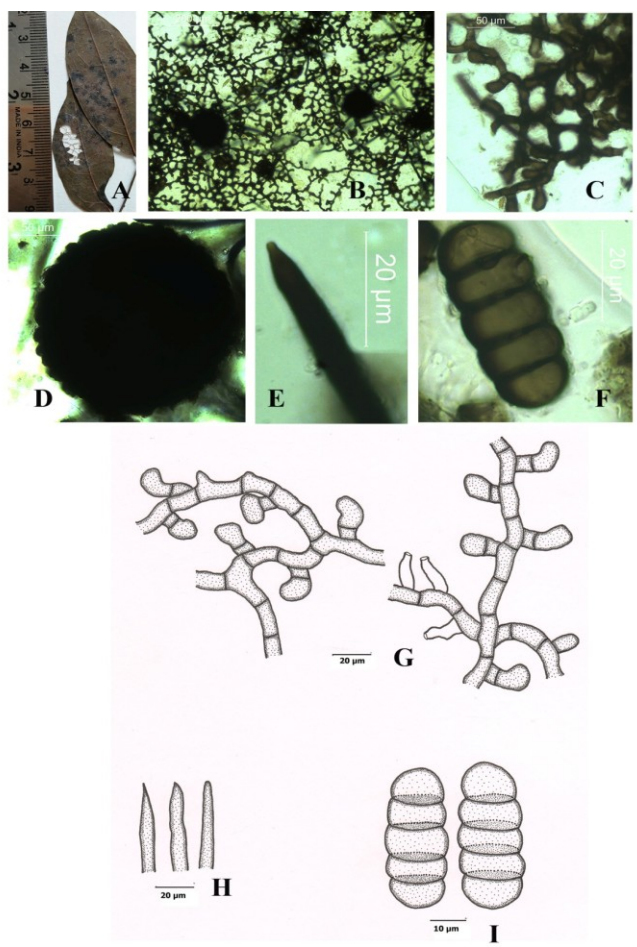

Fig 1. *Meliola roureae* var. *minor*
A. Infected leaves; B. mycelial colony with Perithecia; C, G. Appresoriate mycelium; D. Perithecium; E, H. Apex of mycelial setae; F, I. Ascospores.

Table 1. Comparative account of *Meliola roureae, M .roureae* var. *domingensis and M. roureae* var. *minor* *var. nov.*

Sr. No.	Morpho-taxonomic characters	*Meliola roureae* Syd.	*Meliola roureae* Syd. var.*domingensis* Hansf.	*Meliola roureae* var. *minor var.nov.*
1.	Host plant	*Rourea erecta*	*Rourea surinamensis*	*Rourea minor*
2.	Colonies	Amphigenous	Mostly hypophyllous, up to 4 mm in diam.	Hypophyllous, up to 4 mm in diam.
3.	Hyphae	Substraight, cells 30-40 × 7-9 µm	Crooked, cells 15-30 × 7-10 µm	Undulate to crooked, 14-29 ×7-9 µm
4.	Appresoria	23-30 × 10-17 µm	24-30 × 9-13 µm	14-30 × 11-18 µm
5.	Phialides	20-26 × 8-10 µm	23-29 × 8-10 µm	16-29 × 7-14 µm
6.	Mycelial setae	Up to 1000 µm	Up to 480 µm	Up to 404 µm
7.	Perithecia	Up to 180 µm in diam.	----------	Up to 182 µm in diam.
8.	Ascospores	40-48 × 18 -20 µm	55-65 × 20-22 × 17-19 µm	43-47 16-20 µm

2. *Asteridiella senegalensis* Lonkar, Patil & Salunkhe. *sp. nov.*

MycoBank MB 826814

Beeli Formula: 3101:4230

Etymology: The specific epithet is based on name of the host species.

Type: India, Maharashtra: Bhimashankar Wildlife Sanctuary, on living leaves of *Maytenus senegalensis* (Lam.) Excell.wightiana Babu. (Celastraceae); 21/01/2017, HCIO 52173.

Colonies amphigenous, subdense, dark black, rounded, confluent, crustose, up to 4 mm in diameter. Hyphae substraight, crowded, very closely reticulate, margin crenate, branching opposite, subopposite to alternate at acute angles, cells 9-27 × 7-11µm. Appresoria alternate to unilateral, 1-2% opposite, antrorse, subantrorse, straight, crowded, 13-27 µm long; stalk cell cylindrical to cuneate, 3-9 × 7-11 µm; head cell subglobose, angular, variously lobed, 9-18 × 12-21 µm. Phialides few, ampulliform, opposite, alternate to unilateral, mixed with appresoria but present on separate mycelial branch also, 13-19 × 6-10 µm. Perithecia orbicular, verrucose, grouped at the centre of colony, closely placed, margin crenate, dark black, up to 290 µm. Perithecial wall cells conoid to mammiform, up to 35 µm. Ascospores 4-septate, oblong, end cells are globose, constricted at septa, 41-48 × 18-21 µm.

Habitat and Distribution: Inhabiting living leaves of *Maytenus senegalensis* (Celastraceae) Kondhwal Rai, Bhimashankar Wildlife Sanctuary, Maharashtra, India.

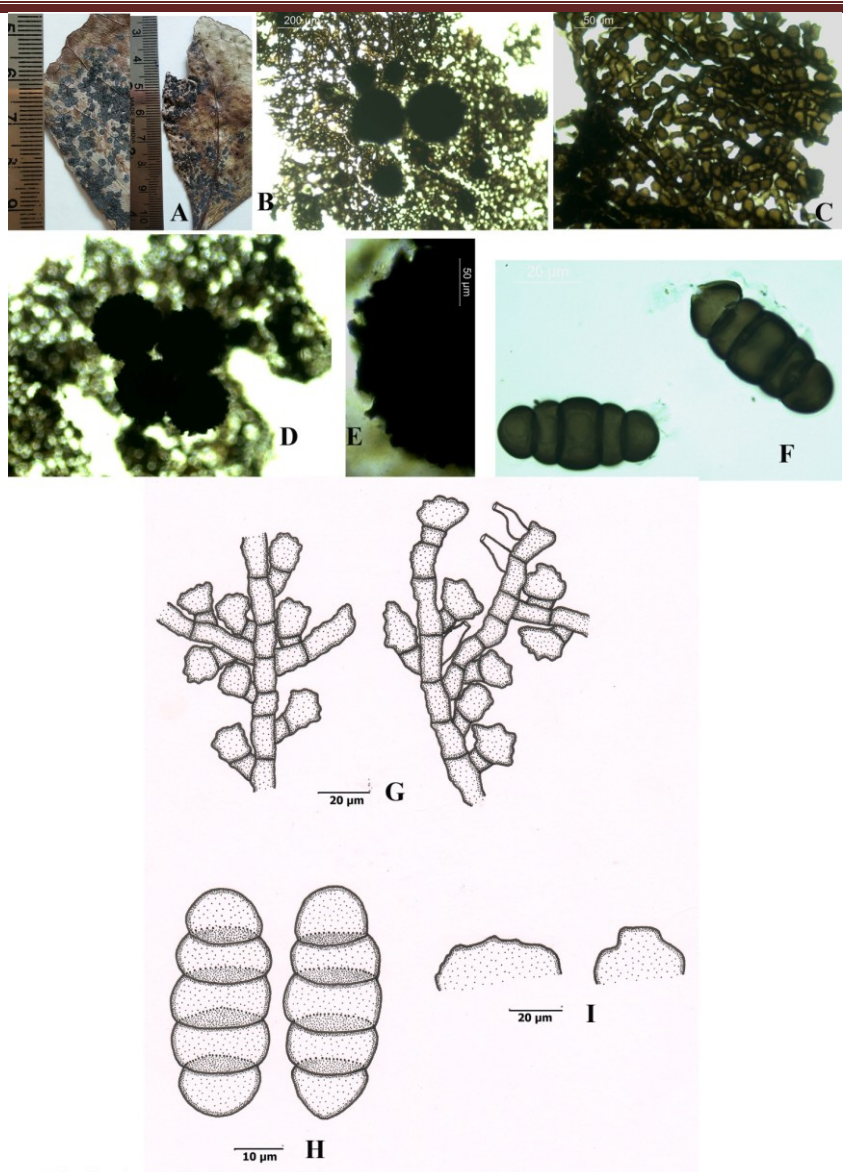

Fig 2. *Asteridiella senegalensis*.
A. Infected leaves; B. Mycelial colony with perithecia; C, G. Mycelium with appresoria & phialides; D. Perithecia; E, I. Perithecial wall cells; F, H. Ascospores.

Table 2. Comparative account of *Asteridiella waimeana, A. perrottetiae, A. gymnosporiae*, and *A. senegalensis* *sp. nov.*

Sr. No.	Morpho-taxonomic characters	*Asteridiella waimeana* Hansf.	*Asteridiella perrottetiae* (Stev.) Hansf.	*Asteridiella gymnosporiae* (Syd.) Hansf.	*Asteridiella senegalensis sp.nov.*
1.	Host plant	*Perrottetia sandwicensis*	*Perrottetia sandwicensis*	*Gymnosporia spinosa*	*Maytenus senegalensis*
2.	Colonies	Amphigenous, up to 1 mm in diam.	Amphigenous, up to 5 mm in diam.	Amphigenous, up to 2 mm in diam.	Amphigenous, up to 4 mm in diam.
3.	Mycelium & Hyphae cell	Substraight, cells 10-15 × 7-9 µm	Substraight to slightly undulate, cells 20-30 × 6-8 µm	Substraight, cells 10-15 × 8-9 µm	Substraight, crowded, margin crenate, 9-27 × 9-11 µm

4.	Appresoria	Straight or bent, head cell versiform, rounded angulose to irregularly lobate, cells 17-28 × 10-18 µm	Straight or bent, head cell versiform, rounded angulose to deepely and irregularly stellate lobate, 20-27 × 10-20 µm	Crowded, straight or slightly bent, head cell subglobose to widely clavate, 12-19 × 10-14 µm	Crowded, Straight, head cell subglobose angular, variously lobate, 13-27 × 12-21 µm
5.	Phialides	Not seen	13-20 × 6-7 µm	--------	14-19 × 6-10 µm
6.	Perithecia	Up to 400 µm in diam, surface cells 20 µm high	Up to 230 µm in diam, surface cells 15 µm high	Up to 180 µm in diam.	Up to 290 µm in diam, surface cells 35 µm high
7.	Ascospores	3-septate, 38-46 × 16-19 µm	3-septate, 37-46 × 13-15 µm	4-septate, 33-40 × 15-18 µm	4-septate, 41-48 × 18-21 µm

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EFFECT OF DASHPARNI LEAF EXTRACT ON GROWTH AND CHEMICAL PARAMETERS OF CHILI (*CAPSICUM ANNUM*L.) VARITY- PUSA JWALA

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ABSTRACT: *In the present work, the effect of Dashparni leaf extract with cow dung and cow urine, on the growth and chemical parameters of chilli was studied. The different concentrations, 50%, 100% and 200% w/v were used to study the effect on the plant height, no. of leaves, fresh weight,dry weight,and biochemical parameters likechl.a,chl.b, total chlorophyll and carotenoid ofchili. It was observed that the lower concentrations of dashparni leaf extract promoted the overall growth of the treated plants as well as increase in the chlorophyll and carotenoid concentrations. However, the higher concentration of dashparni leaf extract was found to be detrimental reducing the growth.*

Keywords: *Dashparni leaf extract, physical parameters, chlorophyll, carotenoid, Capsicum annum L.*

INTRODUCTION-

Chillies, green or red fruits of *Capsicum annuum* L. belong to the family Solanaceae. Chillies are native to Peru and Mexico and Portuguese were the first to introduce chillies in India during 15th century. Its cultivation became popular in the 17th century.

Chilli is one of the most valuable crops of India. The crop is grown largely for its fruits all over the India. Chilli (*Capsicum annum* L) is an important spice cum vegetable crop cultivated in India. (Muduganti R.K.R,2015). It is used in India as a principle ingredient of various curries, and chutneys. It is also used for vegetables, spices, condiments, sauces and pickles. Dry chilies are used for curry powder. Red colour in chili is due to "Capsaicin" which is alkaloid that gives pungency. (www.bighaat.com)

Organic farming is a phrase coined early in the 20th century in reaction to rapidly changing farming practices to describe what other species use, and used, to farm without synthetic chemicals. It relies on fertilizers of organic origin such as compost manure, green manure, and bone meal and places emphasis on techniques such as crop rotation and companion planting. Biological pest control, mixed cropping and the fostering of insect predators are encouraged. In general, organic standards are designed to allow the use of naturally occurring substances while prohibiting or strictly limiting synthetic substances.

The present study is one attempt to prove crop development without harm to the nature. In this study chilli plant was chosen as an experimental plant which was cultivated treating with Dashparni extract to find out the effect on various growth parameters.

MATERIAL AND METHOD :

Dashparni leaf extract is a formulation made of leaves of ten medicinal plant and products from the cow, namely, dung, urine. These products are processed in specific way with a specific ratio and in specific time to obtain best product that is fermented extract.In addition to these jaggery is also used in the preparation of the product. The process of preparation and application of Dashparni is mentioned as below. (Narendra Kumawat et. al. (2014).

Dashparni extract Materials required:

Sr.no.	Plant name	Quantity	Sr.no.	Plant name	Quantity
1	Neem leaves	5 kg	8	*Ricinus communis* (Castor) leaves	2 kg
2	*Vitex negundo* leaves (Lagundi)	2 kg	9	*Nerium indicum*	2 kg
3	*Aristolochia* leaves	2 kg	10	*Calotropis procera* leaves	2 kg
4	Papaya (*Carica papaya*)	2 kg	11	Cow dung	3 kg
5	*Tinospora cordifolia* leaves	2 kg	12	Cow Urine	5 lit
6	*Annona squamosa* (Custard apple) leaves	2 kg	13	green chili paste,	2 kg

7	*Pongamia pinnata* (Karanja) leaves	2 kg	14	jaggery	1 kg

Method of preparation: Crush all above ingredients mentioned in table and mix in 200 lit of water and allow it to ferment for one month. Shake this mixture regularly three times a day. Filter it after one month. This extract can be stored up to 6 months.
Application: Apply this extract with foggerto the plants. The above prepared extract is sufficient for one-acre crop.

OBSERVATIONS :
1. Growth parameters
1.1. Plant height per plant (cm): The plant height was measured from ground level to the terminal growing point of the main stem at 60DAT(days after tratment). The average plant height was expressed in centimeter (cm).
1.2. Number of leaves per plant: Total number of leaves per plant was counted from plants at60 DAT and at harvest and mean was worked out and expressed as number of leaves per plant.
2. Biochemical Parameters
2.1. Estimation of chlorophyll was done by methods given by Arnon (1949).
2.2. Carotenoid was determined according to the method of Lichtenthaler and Welburn (1983).

RESULTS AND DISCUSSION:

The table shows effect on growth and growth attributes:

sr. no.	Treatment		Physical parameter				Chemical parameter			
		Extract conc. and mode of application	plant height in cm	No. of leaves	Fresh wt. in gm	Dry wt. in gm	Chl.a	Chl.b	Chl.a+b	Carotenoid
1	Foliar	T0(control)	68.37	62	32.6	9.6	16.23	23.89	20.13	7.72
		F-T1	70.4	73	34.12	9.8	16.28	25.57	20.17	7.92
		F-T2	52.79	56	26	6.3	16.39	20.51	17.83	6.84
		F-T3	48.63	50	25.62	5.2	15.42	18.35	20.84	3.56
2	Drainage	D-T1	61.68	63	34.11	8.9	19.95	27.86	19.27	7.72
		D-T2	60.96	61	29.3	7.3	18.88	27.71	19.51	4.88
		D-T3	57.45	45	27.64	6.1	18.23	26.35	20.48	4.04
3	Foliar + Drainage	FD-T1	68.73	68	36.41	8.9	26.16	21.15	15.95	8.72
		FD-T2	63	60	34.1	8.9	24.19	25.18	23.56	8.08
		FD-T3	49	50	29.3	6.1	22.47	10.58	16.84	7.16

Table: The effect of Dashparni extract on growth parameters on plant height (cm), number of leaves and fresh wt., dry wt., and chemical parameters of chilli (Capsicum annum L.) cv: Pusa jwala.(conc. of treatments: T1-1:9extract/water, T2-2:8, T3-1:1)

1.Plant growth-At 60 DAT(Days after treatment) maximum average plant height (70.4 cm) per plant was found in foliar application- T1 and minimum was recorded in foliar application of treatment F-T3 (48.63 cm.) where as in dranage application maximum average height of the plant is T1(61.68cm) and minimum is T3(57.45cm).in the same foliar+drainage treatment shows average maximum height is (68.13cm) and minimum height is (49 cm).In control treament height is 68.37cm near about foliar -drainage treatment T0.Pratik Panchal et.al.(2017) reported that the panchagavya enhanced the growth rate of cicer plant and simillar result finding have been reported by Venkatlakshmi et.al.(2009).
2. No.of leaves: At 60 DAT maximum number of leaves was found in foliar application of T1 (73) and minimum was recorded in foliar and drainage application T3 (49).
3. Fresh weight: At 60 DAT maximum weight was found in foliar and drainage treatment T3 i.e.(36.41 gm) whereas in foliar treatment minimum fresh wt.was found i.e (25.62).In control treatment the fresh weight of

plant is (32.6 gm).

4. Dry weight :At 60 DAT maximum dry weight was found in foliar application i.e.T1 i.e.(9.8gm) and minimum weight in foliar treatment was T 3 (5.2gm).in the control the observation was showing T0 is(9.6gm).

Observation on biochemical parameters

Chlorophyll a (mg/g) -At 60 DAT and maximum Chlorophyll a (mg/g) content at was recorded in FD-T1 (26.16mg/g) and minimum was recorded in foliar treatment T3 (15.42 mg/g).

Chlorophyll b (mg/g) -At 60 DAT maximum Chlorophyll b (mg/g) content at was recorded in D-T1(27.86mg/g) and minimum (18.35 mg/g)was recorded in F- T3 .The statistical analysis in table shows that there was significantce.

Chlorophyll a+b (mg/g) - A 60 DAT maximum Chlorophyll a+b (mg/g) content at was recorded in FD-T1(8.72mg/g) and minimum was recorded in F-T3 (3.56mg/g).The statistical analysis in table shows that there was significantce.

Carotenoid (mg/g) -At 60 DAT maximum carotenoid (mg/g) content at was recorded in D- T1 (8.72 mg/g) and minimum was recorded in F- T3 (4.04mg/g). in control it seen T0-7.72mg/g.

CONCLUSION :

On the basis of experiment conducted with Dashparni extractto study its effect on growth of chilli (*Capsicum annum* L.) cv:pusa-jwala it is observed that FT1 shows prominent results with height,no.of leaves and biomass as compared to the controland rest of treatments. the lower Dashpatni extract concentraton promotes growth however the higher concentrations show inhibitory effect on growth of the chilli.

When the effect of treatments on biochemical parameters was studied,it is observed that FD-T1 conc.shows enhanced effect on chl.a,DT1 on chl.b,FDT2 on chl a+b,FDT1 on carotenoidas compared to the control and rest of treatment.

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EFFICACY OF SOME PLANT EXTRACTS AGAINST THE LEAF RUST OF JOWAR UNDER FIELD CONDITIONS

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ABSTRACT: *The effect of plant extracts on leaf rust, Puccinia purpurea Cooke of jowar were studied under field condition in Kharif season 2012. Field application of plant extracts gave effective control on leaf rust disease. Azadirachta indica Juss and Calotropis gigantea L were the most effective treatments gave least percent disease index of 11.88 percent was recorded in T2 followed by T3 recorded 13.73 percent respectively. Least percent disease control of 76.31 percent was recorded in T2 followed by T3 and recorded 71.91 percent. Foliar spray with all plant extracts significantly reduced the percent disease index (PDI). Application of higher concentrations of the extracts showed better disease control. All the treatments were significantly superior to untreated control. Hexaconazole (0.05%) showed complete protection against rust disease incidence in field trails.*

Keywords: *Puccinia purpurea, jowar, hexaconazole, percent disease index*

INTRODUCTION:

Leaf rust disease of jowar caused by *Puccinia purpurea* is considered the most serious disease of jowar. In most major grain-sorghum-producing regions, the disease does not appear until seed is well developed. Hence, grain yield losses are relatively slight when compared to the usual damage caused by cereal rusts (Leukel *et al.,* 1951; Tarr, 1962). However, in warm humid areas of India (Patil-Kulkarni *et al.,* 1972) and in similar areas elsewhere in the world, heavy losses in grain yield occur (Frederiksen, 1980; Miller and Cruzado, 1969). Natural products and non-phytopathogenic fungi, bacteria and yeast have proved to be potential sources of environmentally safe antimicrobial agents useful in plant protection (Eldoksch, 1984; Biles and Hill, 1988; Bar-Nun and Mayer, 1990; Abdel-Moity *et al*., 1993; Eldoksch and Abdel-Moity, 1997; Hassanein and Eldoksch, 1997 and Hammouda *et al*., 1999). Pesticides hazards and resistance problems as well as effects on non-target organisms have produced renewed intrest to naturally occurring pesticides and biocontrol agents. These naturally compounds are often less toxic and less persistent so, they are assumed to be environmentally more acceptable and less hazardous to human and animals.

The present investigation aimed to study the antifungal activity of some plant extracts against the leaf rust, *Puccinia purpurea* of jowar under field conditions.

MATERIALS AND METHODS:

A rust susceptible local variety of jowar was used. A field experiment was conducted during Kharif 2012 in Vahagaon, a village located at the outskirt of Karad city. The experiment was laid out in a randomized block design (RBD) with ten treatments and three replications with size 1x1m plots. The plant extracts that produced high percentage of inhibition at 3% concentration viz, *Capsicum annum* L, *Azadirachta indica* Juss, *Parthenium hysterophorus* L, *Datura stramonium* L, *Calotropis gigantea* (L.) R.Br, *Argemone mexicana* L, *Pongamia pinnata* (L) Pierre, *Nerium oleander* L etc. Extracts of plant parts such as leaf, fruit, seed, etc. were tested further to see their effect *in vivo* conditions.

The uredospore inoculum prepared in tap water was uniformly sprayed in the evening hours to all the treatment plots at 35 days after sowing. In all the treatments totally three sprays were given at 45, 60 and 75 days after sowing. Recommended package of practices were followed to raise the crop. Plant extract of test plants were prepared a fresh on the day of foliar application and used for spray immediately after preparation. The spray treatments were started after 45 days of planting followed by two subsequent sprays at 15 days interval. A standard check with Hexaconazole (0.05%) and untreated control (water spray) was also maintained.

a. Percent Disease Index (PDI)

The five plants were selected from each plot and labeled randomly. The top, middle and bottom leaves of each jowar and were taken, labeled and the index of the disease recorded by scoring all the individual five plants in each cultivar using 0-9 scale (Mayee and Datar, 1986). Further the PDI was calculated with the above scales using the formula of Wheeler (1969).

$$PDI = \frac{\text{Sum of numerical values grades}}{\text{Number of plants observed}} \times \frac{100}{\text{Maximum disease rating}}$$

Where,

PDI= Percent Disease Index.

Spray schedule-

First spray: 45 days after sowing at onset of disease

Second spray: 60 days after sowing first spray

Third spray: 75 days after sowing second spray

Observations on intensity of disease were recorded using five randomly selected plants from each treatment plot and graded as per 0 to 9 scale given by Mayee and Datar(1986). This has been described here under:

Scale

Score Description

0 = No pustules

1 = 1-10% leaf areas covered with rust pustules

3 = 11-25% leaf areas covered with rust pustules

5 = 26-50% leaf areas covered with rust pustules

7 = 51-75% leaf areas covered with rust pustules

9 = > 75% leaf area covered with rust pustules

b. Percent Disease Control (PDC):

The percent disease control was calculated by using the formula of Wheeler (1969).

$$PDC = \frac{\text{PDI in control - PDI in treatment}}{\text{PDI in control}} \times 100$$

Where,

PDC = Percent Disease Control, PDI = Percent Disease Index.

RESULT AND DISCUSSION:

Field experiments were carried out on farms of village Vahagaon during *kharif* season of 2012. The tested plants were found effective *in vitro* used under field condition, for their efficacy against jowar rust. Chemical fungicides Hexaconazole 0.05% was used as standard check and distilled water as a control.

a. Percent Disease Index (PDI) -

The data of disease index recorded at periodic intervals were presented in (table no.01). The treatment differences in respect of index of rust as influenced by plant extracts and fungicide were statistically significant at fifteen days interval of observations except at initial observation (Natural condition). The results indicated that there was a significant difference between the various treatments with respect to percent disease index. Least percent disease index of 11.88 percent was recorded in T2 followed by T3 recorded 13.73 percent respectively. However, these two treatments were superior over rest of the treatments and control. The T10 (Control) recorded maximum percent disease index of 43.28 percent followed by T1, T4 and T5. The T1, T4 and T5 treatments were on par with each other and recorded 19.68, 19.06 and 15.44 percent respectively. The fungicide Hexaconazole 0.05% was least effective (0%) which was followed by control (43.28 percent disease index).

b. Percent Disease Control (PDC) -

The data of disease control recorded at periodic intervals were presented in (table no.02). The treatment differences in respect of disease control of rust as influenced by plant extracts and chemical fungicide were statistically significant at fifteen days interval of observations except at initial observation (Natural condition). The results indicated that there was a significant difference between the various treatments with respect to percent disease control. Least percent disease control of 76.31 percent was recorded in T2 followed by T3 and recorded 71.91 percent. However, these two treatments were superior over rest of the treatments and control. The T10 (Control) recorded less percent disease control of 0% followed by T1, T4 and T5. The T1, T4 and T5 treatments were on par with each other and recorded 58.32, 60.81 and 65.93 percent respectively. The fungicide Hexaconazole 0.05% was maximum effective (100%) and control as less effective in disease control.

CONCLUSION:

Present investigation suggests that locally available plant resources such as *Azadirachta indica* and *Calotropis gigantea* may be of use for possible control of *Puccinia purpurea*. However, further work is needed to explore potential of selected plants at the field level.

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Table-01 Disease index in *Puccinia purpurea* as influenced by different plant extract sprays under field condition at various days after sowing.

Tr. No.	Name of the plant	Plant part used	Mean percent disease index (PDI) at days after sowing			Pooled Mean
			45	60	75	
T1	*Capsicum annum*	Fruit	5.41	15.10	38.54	19.68
T2	*Azadirachta indica*	Leaf	2.45	9.00	24.19	**11.88**
T3	*Calotropis gigantea*	Leaf	2.84	12.17	26.20	13.73
T4	*Argemone mexicana*	Seed	4.46	15.10	37.62	19.06
T5	*Datura stramonium*	Seed	3.63	12.99	29.70	15.44
T6	*Parthenium hysterophorus*	Leaf	6.73	20.23	45.98	24.31
T7	*Pongamia pinnata*	Leaf	8.21	22.21	46.21	25.54
T8	*Nerium oleander*	Leaf	5.66	18.51	42.65	22.27
T9	Hexconazole (0.05%)	Chemical fungicide	00	00	00	00
T10	Control	Distilled water	15.18	39.92	74.76	43.28
SE ±				3.65	0.18	0.24
C.D at 5%				10.95	0.54	0.71

Table-02 : Disease control in *Puccinia purpurea* as influenced by different plant extract sprays under field condition at various days after sowing.

Tr. No.	Name of the plant	Plant part used	Mean percent disease control (PDC) at days after sowing			Pooled Mean
			45	60	75	
T1	*Capsicum annum*	Fruit	64.36	62.17	48.44	58.32
T2	*Azadirachta indica*	Leaf	83.86	77.45	67.64	**76.31**
T3	*Calotropis gigantea*	Leaf	81.29	69.51	64.95	71.91
T4	*Argemone mexicana*	Seed	70.61	62.17	49.67	60.81
T5	*Datura stramonium*	Seed	76.08	67.45	60.27	65.93
T6	*Parthenium hysterophorus*	Leaf	55.66	49.32	38.49	47.82
T7	*Pongamia pinnata*	Leaf	45.91	44.36	38.18	42.81
T8	*Nerium oleander*	Leaf	62.71	53.63	42.95	53.09
T9	Hexconazole (0.05%)	Chemical fungicide	100	100	100	100
T10	Control	Distilled water	00	00	00	00
SE ±						1.43
C.D at 5%						4.30

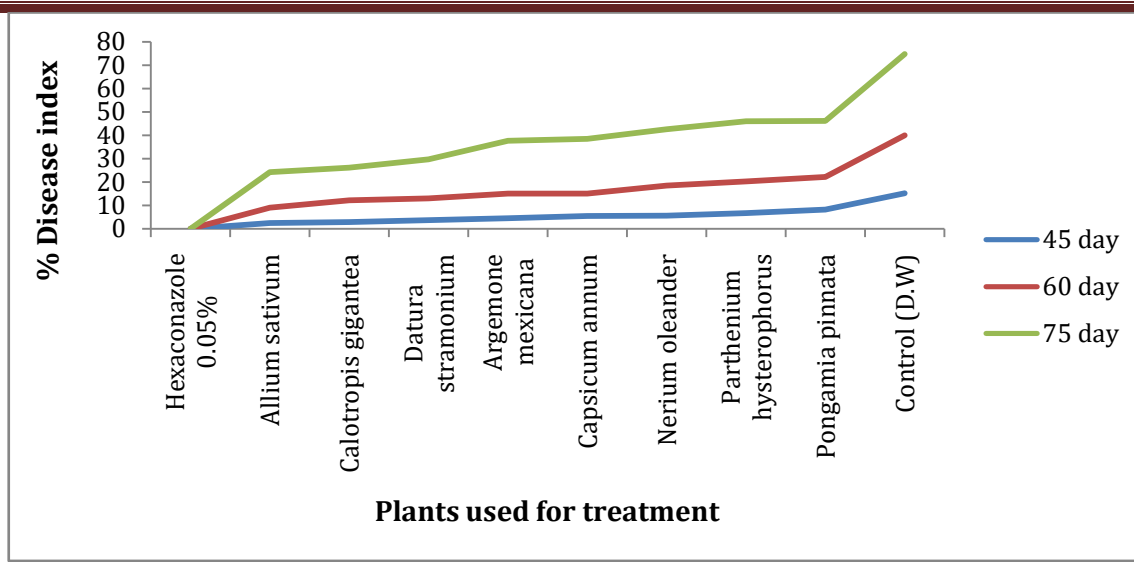

Fig 1- Effect of plant extracts on percent disease index in *Puccinia purpurea*. Data points represent the mean values at 3% concentration after 45, 60 and 75 days incubation period

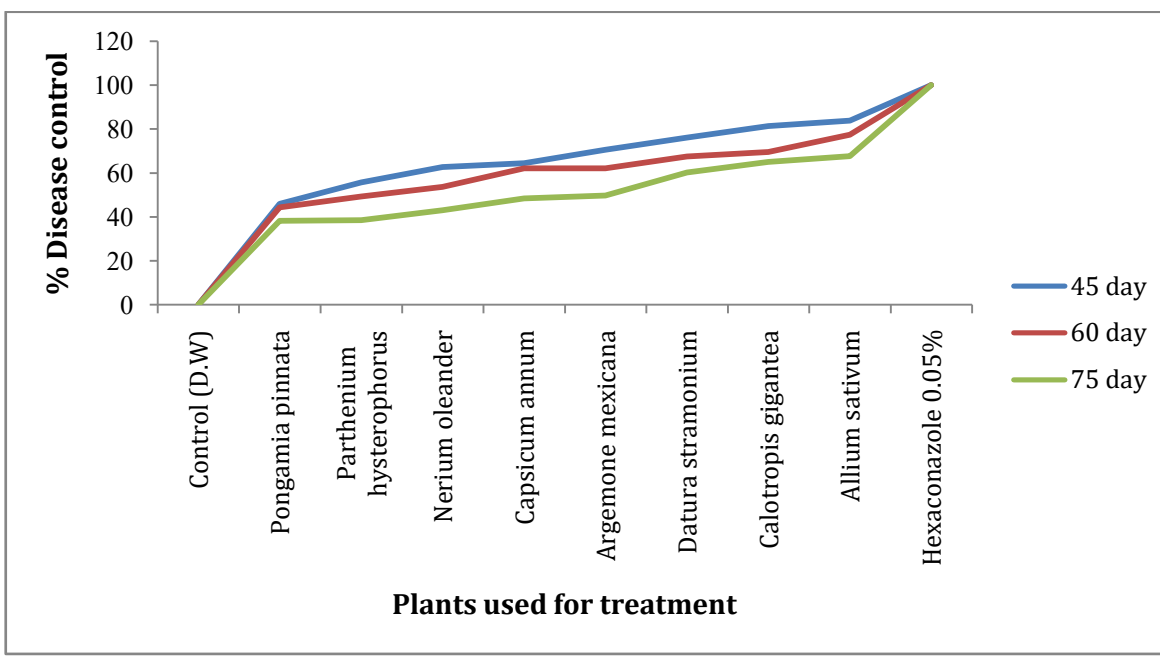

Fig 2- Effect of plant extracts on percent disease control in *Puccinia purpurea*. Data points represent the mean values at 3% concentration after 45, 60 and 75 days incubation period

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STUDIES ON THE EFFECT OF SEAWEED LIQUID FERTILIZER ON SEED GERMINATION AND SEEDLING GROWTH IN VEGETABLE CROPS

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ABSTRACT: *Investigations were made to find out the effect of Seaweed Liquid Fertilizer (SLF) prepared from the green alga Caulerpa racemosa on seed germination and seedling growth of two leafy vegetables namely Spinacea oleracea (spinach) and Trigonella foenum – graceum (fenugreek). The crop seeds were germinated in different concentrations (5%, 15%, 30%, 50%, 70% and 100%) of SLF to record the effect after every 24hrs. The SLF had a promotive effect on seed germination upto 50% in both the vegetables. At higher concentrations there is decrease in germination percentage and seed in growth in both the plants.*

Keywords: *Caulerpa racemosa, Seaweed Liquid fertilizer, seed germination, seedling growth, Spinacea oleracea, Trigonella foenum – graceum).*

INTRODUCTION :

It is known that seaweeds contain plant growth hormones, minerals, vitamins, trace elements and can be used as manure for agricultural crops. The liquid fertilizers obtained from seaweeds is known to promote germination of seeds and increases yields of the crops and also induce resistance to frost, fungal attack and uptake of inorganic nutrients from the soil (Bhosale *et. al;* 1990, Venkataraman *et. al;* 1993, Mohan *et. al;* 1994 and Sekar *et. al;* 1995). Seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds (Dhargalkar and Pereria, 2005).

Among the green algal species, *Ulva, Enteromorpha, Codium,* and *Caulerpa* have been used by a number of workers to investigate their effects on growth and development of crop plants. Green seaweeds used in the form of foliar sprays or applied in the soil, enhanced seed germination, seedling growth and promoted vegetative and reproductive growth thereby increasing the productivity of the crops.

Divya *et al.* (2015) have demonstrated that the SLF of *Ulva* showd a significant increase in the growth of shoot, root, number of leaves, fruit weight, dry weight etc. at lower concentrations. Similarly Francisca and Kalavathy (2011) observed that lower concentrations of SLF of *Ulva lactuca* have stimulatory effect on *Zea mays.* Similar observations were recorded in *Vigna radiata* by Selvam*et al.* 2013. Babu andRengasamy (2012) noticed stimulatory effects in crop plants like *Amaranthus roxburghinus, Arachis hypogea, Capsicuum annum,* and *Tageta serecta* by treatment of *Ulva*.

Sheela and Punitha (2013) observed the effect of SLF of *Ulva fasciata* on *Phaseolus mungo* and reported that the SLF was very effective in promoting root length, number of root nodules, number of fruits and seeds. They also noticed that due to SLF application, concentration of photosynthetic pigments increased. Kasim *et al.* (2015) recorded the influence of extract of *Ulva* on wheat under drought stress. Similar effects were observed on *Solanum lycopersicon* by Hernandez-Herrera *et al.*(2013) using *Ulva lactuca.*Positive effects of *Ulva fenestrata* extract on the length of soybean seedlings were demonstrated by Anismov and Chaikina (2014).

Abhilash *et al.* (2013) studied the effect of SLF of *Caulerpa racemosa* on growth and yield of *Vigna mungo.*They noticed that lower concentrations of SLF promoted germination percentage, radical length, fresh weight, dry weight, productivity, protein and carbohydrate content in the treated plants. All these parameters were adversely affected by SLF at higher concentration. Suganthi and Sujatha (2014) studied the effect of seed treatment and foliar spray of *Caulerpa racemosa* on sunflower and recorded positive effects on plant growth. The promotive effects of *Caulerpa racemosa* extract on growth of black gram were investigated by Sujatha and Vijayalakshmi (2013).

Number of workers have examined the effect of *Caulerpa peltata* on different crops. Paul and Mahadevi (2014) investigated that SLF of *Caulerpa peltata* promoted shoot length, root length, total carbohydrates, total proteins, total lipids, total phenols and total chlorophylls in *Vigna radiata.* Similarly Mahadevi and Paul (2014) studied the effect of *Caulerpa peltata* on germination, shoot length, root length, biochemical constituents and pigment content of *Pennisetum glaucum* and demonstrated that 10% SLF concentration was most effective in promoting the growth.

In the present investigations an attempt has been made to study the effect of Seaweed Liquid

Fertilizer growth on seed germination and seedling growth. The seed germination and seedling growth in two leafy vegetables namely Fenugreek and Spinach was studied using SLF prepared from *C. racemosa.*

MATERIALS AND METHODS :

Preparation of Seaweed Liquid Fertilizer (SLF) :

Green mature thalli of *C. racemosa* were collected from Kunkeshwar situated on west coast of Maharashtra (15.37^0 N to 16.40^0 N, 73.10^0 E to 73.13^0 E) and transported to the laboratory. After washing thoroughly using tap water to remove salts and epiphytes, it was spread on blotting paper to remove excess water.

The SLF was prepared following the method described by Bhosale *et al.* (1975). 1 Kg. of seaweed was cut into small pieces and boiled in 1 liter of distilled water for an hour and filtered through a double layered muslin cloth. The filtrate thus obtained was considered as 100% seaweed liquid fertilizer (SLF). From this desired concentrations (5, 15, 30, 50, and 70%) were prepared by adding proper quantity of distilled water. The diluted SLF were used for seed germination study.

Crop Seeds

The seeds of vegetables selected for the present study were obtained from Shri Ram Krushiseva Kendra, Islampur and Shetakari Sahakari Sangh, Kolhapur. The Crop plants selected for present invegestation are as follows:

Spinacea oleracea (spinach)
Trigonella foenum – graceum (fenugreek).

Germination Studies

Healthy seeds with uniform size, colour and weight were surface sterilized with 0.1% $HgCl_2$ for one minute then thoroughly washed with distilled water and kept in petriplates lined with filter paper and moistened with the desired concentration of SLF. Water soaked seeds were considered as the control. Petriplates were kept in dark for germination at 25 ± 2^0 C germinated seeds were counted and recovered by measuring shoot and root length after 120 h.

RESULTS AND DISCUSSION :

The effect of SLF of *Caulerpa taxifolia* on seed germination and seedling growth in Fenugreek and Spinach is represented in the graph (Fig. 1 & 2) and Table 1.

In *Trigonella foenum-graceum* (Fenugreek) cent-percent seed germination was recorded after 48h with 5%, 15% and 30% SLF. A slight reduction in germination was recorded at higher (100%) concentration of SLF. In *Spinacea oleracea* (Spinach) upto 50% concentration, the treatment was effective and 100% germination was seen after 120h at 30% SLF concentration. In untreated control seeds maximum percent of germination achieved was 95 even after 120h. Reduction in germination was recorded at 70% and 100% SLF.

In the present study maximum seedling growth was recorded at 30% SLF concentration in both the plants. In both the crops shoot length was promoted upto 50% SLF concentration. A slight decrease in shoot length was recorded at 70% in Spinach. In both the crops shoot length was decreased at 100% SLF. In Spinach, root length increases upto 30% SLF, while in Fenugreek root length increases upto 70% SLF concentration and a slight decrease in root length was recorded at 100% SLF. In case of Spinach, decrease in root length was recorded at 70% and 100% SLF.

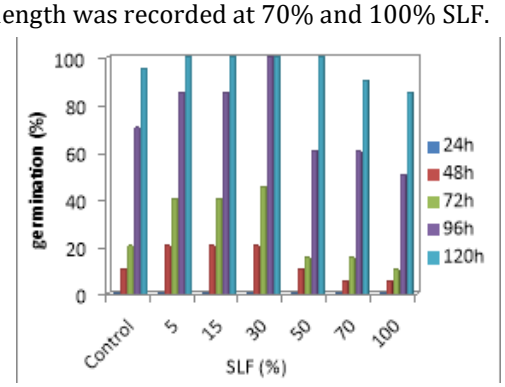

Fig. 1: Effect of SLF of *C. racemosa* on seed germination in *Spinacea oleracea*

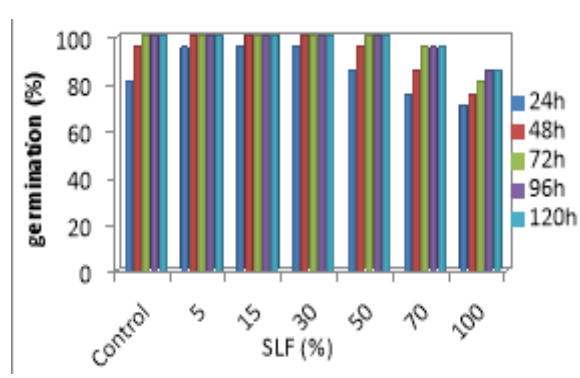

Fig. 2: Effect of SLF of *C. racemosa* on seed germination in *Trigonella foenum-graecum*

Table 1 : Effect of SLF of *Caulerpa racemosa* on seedling growth in *Spinacia oleracea* (Spinach) and *Trigonella foenum-graecum* (Fenugreek)

SLF (%)	*Spinacia oleracea*		*Trigonella foenum-graecum*	
	SL	RL	SL	RL
Control	5.04	3.52	5.05	2.94
5	5.62	3.81	6.48	3.99
15	5.88	4.22	6.92	4.29
30	6.62	4.38	6.99	4.96
50	5.08	2.92	5.29	3.37
70	4.83	2.85	4.51	3.18
100	4.2	1.42	3.99	2.92

CONCLUSION :

In the present study an attempt has been made to study the effect of SLF of *Caulerpa racemosa* on seed germination and seedling growth in two leafy vegetables. From present investigation it can be concluded that SLF of *Caulerpa racemosa* possess fertilizer activity to enhance the germination and seedling growth in two leafy vegetables. It is probably due to presence of growth promoting hormones. SLF are economical and ecofriendly alternatives to the chemical fertilizers.

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PROXIMATE ANALYSIS FOR NUTRITIONAL POTENTIAL OF EDIBLE MANGROVE FRUITS OF *SONNERATIA APETALA.*BUCH-HAM FROM DEVGAD.DIST. SINDHUDURG

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ABSTRACT: *The proximate analysis of fruit of Sonneratia apetala revels that, fruit of S. apetalacontains, total minerals(04.11%), Crude protein (06.16%), crude Fat (02.68%) and Crude Fiber (18.56%), Carbohydrates (57.28%), and energy (277.88Kcal/100g). It is revealed that fruits of Sonneratia apetalacontains significant amount of Moisture, Total minerals, Crude protein, crude Fat, and Crude Fiber, Carbohydrates, and energy, these fruits are eaten by monkeys hence these fruits might be useful as a source of nutrients for human beings to fulfill the need of malnutrition of the local people, after detailed study.*

Keywords: *Proximate, Moisture, Crude protein, crude Fat, Crude Fiber, Carbohydrates, energy.*

INTRODUCTION:

Mangrove ecosystem forms an important element of coastal environment. Among different mangrove species growing in kokanregion of Maharashtra, *Sonneratia* is an important species. According to Bhosale (2005), three species of *Sonneratia* occur in the mangrove vegetation of kokan region. These are namely *Sonneratia alba*, *Sonneratia caseolaris*, and *Sonneratia apetala*belongs to family Lythraceae. Kurlapkar (1983) reported that, species *Sonneratia apetala* is only present in India, Sri Lanka, and Ritchie's Archipelago,Burma.He indicated that *Sonneratiaapetala* is an important component of mangrove flora of Sindhudurg District.

Many research worker reported that, mangrove ecosystems have always providing abundant nutritious food to local people of that region, and constituting an important source of income for mangrove populations. Schaeffer-Novelli, (2003) reported thata mangrove ecosystem is an important source for protein and stated that,great part of protein consumed by local people comes from the mangrove ecosystem, where these populations practice self-maintenanceextractivism.He stated that, fruits of different mangrove speciesplays an important and a substantial role in the food and nutrient safetyin general of coastal people community, especially of the rural poor.Fruits are nutritionally rich and provide supplement nutritional requirements for the forest societies and many of the demoted rural communities subsequently the common cultivar fruits are less familiar and not accessible for them. In view of the ever-increasing problem of human population and reducing natural resources, there is a requirement to achievement the role of mangrove eatable fruits to the fullest amount possible. To the contrary, the mangrove fruits, which is used by the local communities, are not the aware to the urban populations.Information accessible on the eatable as well as beneficial properties of the mangrove fruits is requisitioned and data on their nutrition aspect are inadequateorinsufficient. During recent years, there has been an increasing interest to estimate various mangrove fruits for their nutritional value [Sudirman*et al*., (2015), Patil and Chavan(2013)].

Some studies have previously carried out on the nutritive values and presence of potent micronutrient in the fruits of different plant species (Bellec et *al*., 2006). But less studies have been documented with fruits of mangrove plants (Halder,*et al*., 2013). Besides, providing nutritional properties, these mangrove edible fruits also can serve as natural antioxidant. Fruits are rich with antioxidants that help in lowering incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Feskanich*et al*., 2000). Some reports are available i.e*Bruguiera gymnorrhiza* fruits (Sudirman*et al*., 2015), *Sonneratia caseolaris* fruit (Santoso*et al.,* 2011) but reports on *S. apetala* fruit still lacking. The present piece of work explores the nutritional status of edible mangrove fruits of *S. apetala*with a view to assess some promising species which may be considered as non-conventional bio-nutritional sources based primarily on their nutritional properties like Crude protein, crude Fat, Crude Fiber, Carbohydrates , and energy of mangrove fruits.

MATERIAL AND METHODS:

Plant material fruits of *S. apetala*were collected from IlyesadaTal. Devgad. Dist. Sindhudurg. Fruits were washed thoroughly and blot to dry with clean and sterilized cotton cloth. Then the Plant material was

dried in oven at the temperature 60°c for 10 days. Dried material was ground finely to form a powder with the help of Willey's grinding machine. And brought to recognized laboratory, Nikhil analytical research laboratory Sangli for further analysis. In laboratory we analyzed, Total carbohydrate content, crude proteins, moisture, total minerals, crude fats, crude fiber, and energy.

1. Total carbohydrate content: The 0.2g oven dried, fruit powder of *S. apetala*was subjected to extraction, with 80% neutral alcohol and determined according to (Nelsons 1944).

2. Total protein content: For determination of protein,in the fruit of *S. apetala*the standard method described by (Lowry *et al.,* 1951) was used. As a standard, Bovine serum albumin (BSA) was used and total protein content was expressed in mg/100g.

3. Total energy content: For determination of total energy,the standard method described by (Brett and Groves, 1979) was used. The energy content of fruit of *S. apetala*was determined by multiplying the values obtained for protein, carbohydrate and lipid by 23.86, 17.16 and 36.42 kjg^{-1} respectively.

4. Total lipid content : For determination of total lipid content,the standard method by (Folch*et al.,* 1951) was used. The lipid content of fruit of *S. apetala*was determined by extracting 0.5 g of homogenate with chloroform: methanol (2:1 v/v). Lipid extract was collected and solvent was evaporated with a rotary evaporator. The residue was considered as total lipid content, and measured gravimetrically.

5. Total dietary fiber: For determination of total dietary fiber content,the standard method by (DeVries and Rader 2005) was used. The dietary fiber content of fruit of *Sonneratia alba* was determined by formula,

$$\textbf{Total dietary fiber} = \frac{[\text{Weight residue} - \text{protein} - \text{ash} - \text{blank}]}{\text{Weight test portion}}$$

RESULT AND DISCUSSION :

Proximate configuration of food is used to study the estimation of the nutritive worth of human food in the chemical form. The percentage of moisture content, Total minerals, crude protein, Crude fat, Crude Fiber, carbohydrate and energy in the plant parts showed slight variation between samples and species. The proximate analysis of fruits of mangrove species *Sonneratia apetala*is given in *Table1*.

The moisture content of fruits is 11.21% in dry fruit powder of *S.apetala.*Total minerals in fruit of *Sonneratia apetala* is 04.11%, Crude protein in fruit of *Sonneratiaapetala* is 06.16%.Proteins are made up of carbon, hydrogen, oxygen and Nitrogen. These are complex compounds with high molecular weight. Protein molecules are made up of long chain of Amino acids. Proteins are essential for formation of structure of cell as well as these are essential for formation of different types of enzymes, hence proteins acts as structural as well as catalytic role. Gururaja Rao *et.al*,(1999) and other many research workers have reported proteinsensitivity in mangroves.Rao (1974) reported that protein content decline with age in *Sonneratia apetala.* Crude Fat in fruit of *Sonneratia apetala*is 02.68%. Crude Fiber in fruit of *Sonneratia apetala* is 18.56%, Food rich in dietary fiber subsidizes to the inhibition of various diseases such as, most of the spreading diseases like diabetes, constipation, colon cancer, hemorrhoids, diverticulosis, and , excess cholesterol. Carbohydrate in fruit of *Sonneratia apetala*is 57.28%.Carbohydrates are main reserve organic compounds in the plants. In initial stage of growth of plants, carbohydrate is a main source of nutrients. Ackerson and Younger (1975) reported that,mangroves tolerate in the saline condition due to three mechanisms, they were further stated that, carbohydrate is one among three mechanism. It is thought that in mangrove, carbohydrates take part in osmoregulatory process.Energy content in fruit of *Sonneratia apetala* is 277.88kcal/100gm., it indicates that energy content in fruit of *Sonneratia apetala* is higher as compared to other mangrove plants.

CONCLUSION :

Fruits of *Sonneratia apetala* Buch-Ham. plant shows significant amount of Moisture, Total minerals, Crude protein, crude Fat, and Crude Fiber, Carbohydrates, and energy. Hence fruits are useful as a source of food. Further study is necessary.

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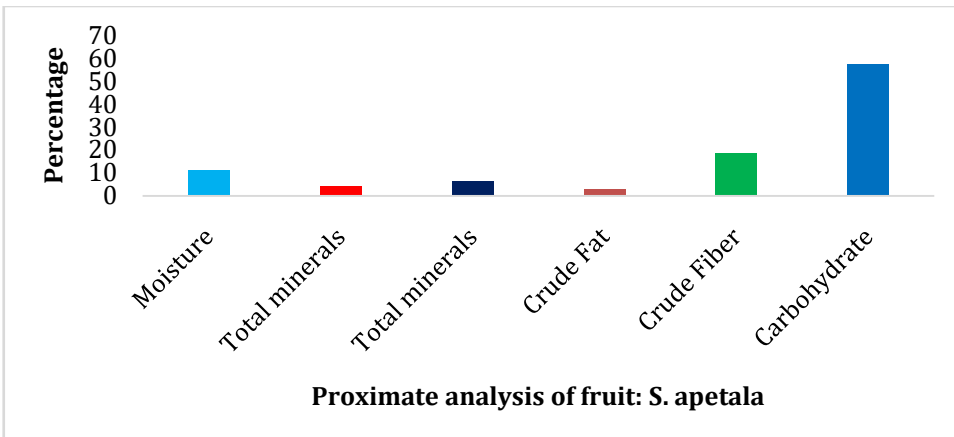
Table 1: Study of Proximate analysis of Fruit of *Sonneratia apetala* Buch-Ham.

Sr. no	Parameter	Unit	*S. apetala*
1.	Moisture	%	11.21
2.	Total minerals	%	04.11
3.	Crude protein	%	06.16
4	Crude Fat	%	02.68
5	Crude Fiber	%	18.56
6	Carbohydrate	%	57.28
7	Energy	Kcal/100g	277.88



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REGRESSION OF INDIAN VEDAS IN SUSTAINABLE ENVIRONMENT

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ABSTRACT: *The Vedas are the primary sources of not only moral enhancement for the economic man but also paths for misguided ecology to achieve a true sustainability. It views human perfection and happiness from integrated perspectives, which embraces both material and spiritual values in individual and harmonious unity. The Vedas will guide to enlighten the inner human soul in order to maintain have moral values, true purpose of life and care for nature. The Vedic cultures are unique from development perspectives.*

The present crisis of environment degradation is the direct result of this merciless exploitation and ravishment of nature. As long as a sense of love and respect for nature is not developed, all materialistic efforts of environment protection would remain superficial and futile.

Every country of the world has showed a deep concern in the protection of the environment. But this concept of the protection of the environment was already present in Vedic literature. In Vedic literature we find that during the Vedic period the society was very much concerned about the protection of the environment. But during the last century unfortunately in the name of industrial development our environment becomes degraded in a very alarming rate. The ancient Vedas have several references on environmental protection, ecological balance, weather cycles, rainfall phenomena, hydrologic cycle and related subjects that directly indicate the high level of awareness of the seers and people of that time.

The Vedas have the potential of many remedies to the today's world problems of unsustainable development. For instance, "Dharma" is typical world in Hindu literature including VEDAS and still in the Vedic cultures. It implies the sustainability, that is, if anyone violates the dharma, God may punish the violator, so no one will or should act against it. People save or even earn the dharma by visiting the temple or holy places in order to pave the way for heaven and have liberation, despite the multitude of sinful acts in other places. It seems that the modern act of Hindu people is also deviating from the Vedic philosophy, which is primarily due to the influence of "economic man".

INTRODUCTION :

Vedas are universally accepted to be the most precious indian heritage. The Vedas have knowledge of all types and the main vedic views revolve around the concept of nature and life. Vedas contained several references on environment conservation, ecological balance, and weather cycle. This indicates the high level of awareness of the people at that time. In ancient india, protection and cleaning up of environment was the essence of vedic culture in hindu philosophy forests, trees and wildlife protection held a place of special respect. Cutting green trees was prohibited and punishment was prescribed for such acts.

The Vedas attach great importance to environmental protection and purity. They persist on safeguarding the habitation, proper afforestation and non-pollution. In fact, man is forbidden from exploiting nature. He is taught to live in harmony with nature and recognize that divinity prevails in all elements, including plants and animals. The rishis of the past have always had a great respect for nature.

VEDAS- Type :

The **Rigveda** (means Knowledge of praise) is an old Indian gathering of Vedic Sanskrit hymns. It is one of the four accepted consecrated writings of Hinduism known as the Vedas. The content is an accumulation of 1,028 hymns and 10,600 verses, sorted out into ten books (Mandalas). A decent arrangement of the dialect is as yet dark, and numerous songs as a result are muddled.

The **Yajurveda** (knowledge of composition mantra) is the Veda of exposition mantras. An old Vedic Sanskrit content, it is an arrangement of custom offering recipes that were said by a cleric while an individual performed custom activities, for example, those before the yajna fire. Yajurveda is one of the four Vedas, and one of the sacred texts of Hinduism. The correct century of Yajurveda's sythesis is obscure, and evaluated by researchers.

The **Samaveda** (knowledge of song) is the Veda of melodies and chants. It is an ancient Vedic Sanskrit language text, and part of the scriptures of Hinduism. One of the four Vedas, it is a liturgical text which consists of 1,549 verses. Except 75 verses rest have been taken from the Rigveda. Three recensions of the Samaveda have survived, and variant manuscripts of the Veda have been found in various parts of India.

While its earliest parts are believed to date from as early as the Rigvedic period, the existing compilation dates from the post-Rigvedic Mantra period of Vedic Sanskrit, c. 1200 or 1000 BCE, but roughly contemporary with the Atharvaveda and the Yajurveda.

The **Atharva Veda** is the "knowledge storehouse of *atharvaṇas*, the procedures for everyday life". The text is the fourth Veda, but has been a late addition to the Vedic scriptures of Hinduism.

The Atharvaveda is composed in Vedic Sanskrit, and it is a collection of 730 hymns with about 6,000 mantras divided into 20 books. About a sixth of the Atharvaveda text adapts verses from the Rigveda, and except for Books 15 and 16, the text is in poem form deploying a diversity of Vedic matters.

VEDIC SCIENCE - The pursuit of truth :

The universe we live in is a multidimensional reality from the subatomic to the supragalactic in the realm of physics alone. Biology, medicine, psychology and the social sciences require different perspectives and approaches to deal with appropriately. On top of these are subtle forces and influences, extrasensory, occult and spiritual that many people claim to experience as well and have developed special methods of working with.

Real science consists of an objective pursuit of truth through observation and experimentation. It occurs apart from any beliefs or preconceptions about what it is going to find. It is based upon reason and direct perception, in which the reality is allowed to reveal itself to the unbiased eye.

Vedic science urges people to pursue the path of ethical and sustainable economy, which coincides with the philosophy of ecological economics for sustainable development. The conventional economics always favors maximizing the material wealth so that individual will have a better quality of life.

VEDA AND ECOLOGY :

Earth is represented as goddess, which feeds everyone. All the forms of earth and of life on it are the children of earth. Rivers, trees animals are attendants of the earth goddess. The sun, moon, rain, wind and lightening are the children of sky. The sun is one of the three main deities of Vedas, which is at the center of creation and known as the nourisher.

The seers of Rig Veda on behalf of the earth for its principle of replenishment "You give me and I give you". They look at every entity of Nature with the eye of a friend and sympathizer. Mitrasyaaham Chakshushaa Sarvaani Bhootani Sameekshe". The Rig Veda makes a clear reference to the presence of a protective layer 'which we know now to be the ozone layer' that filters the harmful rays of the sun and protects the earth and praises the radiation that enters the atmosphere that is responsible for the health of environment. In a hymn of Rig veda the seer prays to the Ashvin for their indulgence for protection against any excessive solar flare that also affect earth's temperature.

The Vedas viz. Rig, Sama, Yajur and Atharva Vedas perceive the importance of maintenance of the season's cycles that are likely to get altered due to the climate change owing to inappropriate human actions. It is noticeable that the people in Vedic Period regarded Nature and the Environment in a holistic manner and revered each of its constituents and entities by carefully preserving them. Respect to the nature and to all natural resources such as land, water, hills. Forest, other animals etc. was the first message of Vedic philosophy. Environment has been perceived as a friendly abode in the Vedic literature. For any inadvertent action leading to earth's excessive exploitation the seers prayed for forgiveness, 'Whatever I dig from thee, O Earth, may that have quick recovery again. O purifier, may we not injure thy vitals or thy heart', (Hymn No- 12 slok No.- 34, Atharva Veda, Prithvi Sukta). The text of vedic period always created a greater awareness regarding the conservation of water which is one of the most important environment resources. Because of its indispensable nature it is to be of divine nature and the following Rigveda verse (VII: 49: 2) is a good example of this idea " the water from heaven, the water from the spring, the bright pure water which tends to the sea, may these divine waters protect us here." The significance of water for life was well-known to Vedic seers. They knew the water cycle that can be perceived by the following hymn- " Waters from the ocean to the sky they carry up, they who pour from the sky upon the earth…"(Atharva Veda 4:27:4). They mention -Waters are nectars. Waters are source of all plants and giver of good health. Waters destroy diseases of all sorts. Waters are for purification. It seems that later developed cultural tradition of pilgrimage on the river-banks is based on the theory of purification from water. The ancient Indians knowing water as a vital element for life were very particular to maintain it pure and free from any kind of pollution.

VEDA AND BIO-DIVERSITY :

The Vedas and Upanishads mention that the gods and goddesses favour different biological resources. Knowledge of biodiversity, interrelation between living species and the environment, the need to maintain natural dynamism, and the natural way of transgressing the ecological principles are mentioned in **Yajur-veda.**

Similarly the Rig-Veda mentions about the forest goddess and healing properties of plants, tribes of fishes, goats, and etc. Cow is believed as theomorphic animal in **Atharva-veda.** Hence, followers of Veda should avoid eating it.

There are many sacred plants that Hindus worship regularly. For instance Tulsi, Rudrakchya, Bar, Pipal and Sami are the most religious plant species. IN Rig Veda Soma is mentioned as king of plants there are hundreds of medicinal plants which are in use from Vedic period to now. This is one of the main economic activities i.e. harvesting of wild medicinal plants, of people living in the upper mountain areas of Nepal.

There are some ecological codes of conduct practiced among the followers of Vedas. One should always pray to the food. No one should disturb the habitat of wild animals. Also trees should not be disturbed during night because of presence of gods' soul in the tree trunk. Such rules are mentioned in the law of MANU (MANUISMRITI) and further catalogued by the Tiwari and Dwivedi.

According to the activist *vandana Shiva's* book, the seed keeper, new seeds were firs worshipped before being consumed. New crop was worshipped before being consumed. For the farmer, field is the mother; worshipping the field is a sign of gratitude towards the earth, who as mother feeds the millions of life forms who are her children.

A typical rice field supported and in some places continue to do so 800 species of "friendly insects", spiders, wapes, ants and pathogens that controlled 95% of insect pests.

These practices are still a living presence among India's tribal societies, for instance, the Warlis, a community near Mumbai worship nature of Hirva (green) and consider all produce to be gifts of Hirva, rather the fruits of their own labour.

Conservation of plants and animals was an innate aspects of Veda culture, illustrated in the concept of the source of grooves; mangroves, marshlands and other tracts of land supposedly inhabited by spirits, where killing of plants and animals is taboo.

VEDA FOR SUSTAINABLE ENVIRONMENT :

The Vedas have the potential of many remedies to the today's world problems of unsustainable development. For instance, "Dharma" is typical world in Hindu literature including Vedas and still in the Vedic cultures. It implies the sustainability, that is, if anyone violates the dharma, God may punish the violator, so no one will or should act against it. People save or even earn the dharma by visiting the temple or holy places in order to pave the way for heaven and have liberation, despite the multitude of sinful acts in other places. It seems that the modern act of Hindu people is also deviating from the Vedic philosophy, which is primarily due to the influence of "economic man".

It may sustain for a while, but not for the long term or strong sustainability. Hence, people need to have the real dharma, which loves nature and does not wish to rule over the nature, should be today's ideal philosophy for development.

The present crisis of environment degradation is the direct result of this merciless exploitation and ravishment of nature. As long as a sense of love and respect for nature is not developed. All materialistic efforts of environment protection would remain superficial and futile.

The Vedas attach great importance to environmental protection and purity. They persist on safeguarding the habitation, proper afforestation and non-pollution. In fact, man is forbidden from exploiting nature. He is taught to live in harmony with nature and recognize that divinity prevails in all elements, including plants and animals. The rishis of the past have always had a great respect for nature.

Hinduism recognizes that the human body is composed of and related to these five elements, and connects each of the elements to one of the five senses. The human nose is related to earth, tongue to water, eyes to fire, skin to air and ears to space. This link between our senses and the elements is the foundation of our human relationship with the natural world. For hinduism, nature and the environment are not outside us. They are an inseparable part of our existence.

A verse from rig-veda says, "thousands and hundreds of years if you want to enjoy the fruits and happiness of life then take up systematic planting of trees." The term pollution did not exist at that time but

they call it poisoning of environment. They believe that the five great elements (space, air, fire, water and earth) that constitute the environment are all derived from *prakriti*, the primal energy and our human body is composed of these and related to these five elements, and connects each of the elements to one of the five senses. The human nose is related to earth, tongue to water, eyes to fire, skin to air and ears to space. This bond between our senses and the elements is the foundation of our human relationship with the natural world. For hinduism, nature and the environment are not outside us. They are an inseparable part of our existence, and they constitute our very bodies. The Vedas stress the need for protection and development of forests. Human beings have to safeguard the trees. The v emphasize that the plants and trees are the treasures for generations. It is amazing that the people in vedic times regarded nature and the environment in a holistic manner and revered each of its constituents and entities by carefully preserving them. "do not harm the environment; do not harm the water and the flora; earth is my mother, i am her son; may the waters remain fresh, do not harm the waters".“do not cut trees, because they remove pollution.” (*rig veda,* 6:48:17)“do not disturb the sky and do not pollute the atmosphere.” (*yajur veda*,5:43) besides Vedas, upanishads, puranas, sutras and other sacred texts of hinduism contains a number of references of the worship of the nature. Our sanskrit mantras daily remind us that our rivers, mountains, trees, animals and the earth deserve respect and dignity. The upanishads are a collection of texts that contain some of the central philosophical concepts of hinduism, some of which are shared with buddhism and jainism.

Vedas are universally accepted to be the most precious indian heritage. The Vedas have knowledge of all types and the main vedic views revolve around the concept of nature and life. Vedas contained several references on environment conservation, ecological balance, and weather cycle. This indicates the high level of awareness of the people at that time. In ancient india, protection and cleaning up of environment was the essence of vedic culture in hindu philosophy forests, trees and wildlife protection held a place of special respect. Cutting green trees was prohibited and punishment was prescribed for such acts.

Environmental degradation is a great problem of the modern world. Protection of Environment is a burning problem all over the world. Every country of the world has showed a deep concern in the protection of the environment. But this concept of the protection of the environment was already present in Vedic literature. In Vedic literature we find that during the Vedic period the society was very much concerned about the protection of the environment. But during the last century unfortunately in the name of industrial development our environment becomes degraded in a very alarming rate. The ancient Vedas have several references on environmental protection, ecological balance, weather cycles, rainfall phenomena, hydrologic cycle and related subjects that directly indicate the high level of awareness of the seers and people of that time.

The Rig Veda explains deities' viz. Mitra, Varuna, Indra, Maruts and Aditya that are responsible for maintaining the balance in functioning of all entities of nature like hills- mountains, lakes, heaven and earth, forest or the waters. The Indra, Surya and Agni are the personification of the Watery atmosphere, the source of heat and light and fire. Likewise the dawn, the wind, the assemblage of moving power and some of the natural phenomenon are personified as Ushas, Vayu and Maruts etc. Vedas perceive that any change in the nature caused due to indiscreet human activities could result in imbalance in weather, rainfall patterns, and crops and may pollute earth, air and water. There are so many hymns seeking the blessings of the five gross elements of life or the Pancha mahabhoota of nature: akasha or firmament, vayu or air, tezas or fire, apahh or water and prithvi or earth. People were careful to refrain themselves from any activities which could cause harm to the nature's bounties. It was understood that the well-being of Mother Earth dependant on the preservation and sustenance of the environment.

In the AtharvavedaVeda (Prithvi Sukta, slok no.- 12), the Vedic seer solemnly declares the enduring filial allegiance of humankind to Mother Earth. “Mata Bhumih Putroham Prithivyah” : Earth is my mother, I am her son.'. It can be understood that Vedic society was the first Environmental Protection Society in the history of mankind. In Veda we find the concept that everything in the world has life in it whether it is living or non-living. Veda begins with the worship of Gods of Nature, viz. the terrestrial Gods like Prithvi, Agni, Brihaspati and Soma, the atmospheric Gods like Indra, Rudra, Maruts, Vayu and Parjanya and the celestial Gods like Dyaus, Varuna, Ushas and Asvins. Thisclearly reveals that in vedic period the Humankind had clear concept of their surroundings.

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COMPARATIVE STUDIES ON PHENOLIC, FLAVONOIDS CONTENT AND ANTIOXIDANT ACTIVITY OF *TRIDAX PROCUMBENS* L.

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ABSTRACT: *Tridax procumbens L. is a herbaceous trailing weed found throughout India. Tridax procumbens L. is later on reported from tropical, subtropical and temperate regions of the world but originally it is native to tropical America. Tridax procumbens L. (Family- Compositae), is a traditional medicinal herb, it has exllent medicinal value. It is best known as widespread weed. The present study was carried out by total phenolic content, total flavonoid content and DPPH radical scavenging activity. They were determined in leaves of Tridax procumbens L. The total phenolic content during monsoon season has been recorded as 88.02 ± 0.12 % and the total flavonoid content as 47.32 ± 0.87mg/100 gm. in the methanolic extract of this plant. The DPPH radical scavenging activity in investigated sample was 48.24 ± 0.32 in mansoon season 2017.*

Keywords: *Tridax procumbens L., total phenolic content, DPPH, total flavonoid content.*

INTRODUCTION :

India is a country with a vast reserve of natural resources and a rich history of traditional medicine. The different systems of medicinal usage practiced in India, Ayurveda, Siddha, Unani, Amchi, Homoeopathy and local health traditions, utilize a large number of plants for treatment of human and animal diseases. Those plants used were called as medicinal plants" (Gaikwadi et al., 2003).*Tridax procumbens* L. is a species of flowering plant growing as a common weed. It is found in America, West Africa and other tropical zones of world including India, classified under the daisy family Asteraceae. It is known coat button in English, jayanti Veda in Sanskrit, Ghamra in Hindi and Dagadi-pala in Marathi. The plant bears white or yellow flowers with three toothed ray florets. The leaves are toothed and generally anchor shaped. Its fruit is hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. Calyx is represented by scales or reduced to pappus."*Tridax procumbens* L. has many medicinal properties, such as immunomodulatory, anti-diabetic, antihepatotoxic, antiviral, antioxidant, antibiotic efficacies, wound healing, insecticidal, parasiticidal, anti-inflammatory activity, prevention of bleeding, bronchial catarrh, diarrhea, dysentery, etc."(Jain et.al., 2012). Medicinal plants contain important natural antioxidants traditionally used as medicines for thousands of years which are used in herbal preparations of Ayurveda. It is an important component of Bhringraj an Ayurvedic preparation. "Several secondary metabolites were isolated from the plants which are used as antimicrobial agents.

Scientific classification-

Kingdom: Plantae
Sub-kingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Sub-class: Asteridae
Order: Asterales
Family: Asteraceae
Genus: Tridax
Species: Procumbens

Botanical name: *Tridax procumbens* Linn.

MATERIAL AND METHOD :

Plant material collection and Authentication

The fresh leaves *Tridax procumbens* L. were collected in the Vetal hill, District -Pune (MS), India in mansoon season 2017. The plant was authenticated by Botanical Survey of India (BSI) Pune as *Tridax procumbens* L.

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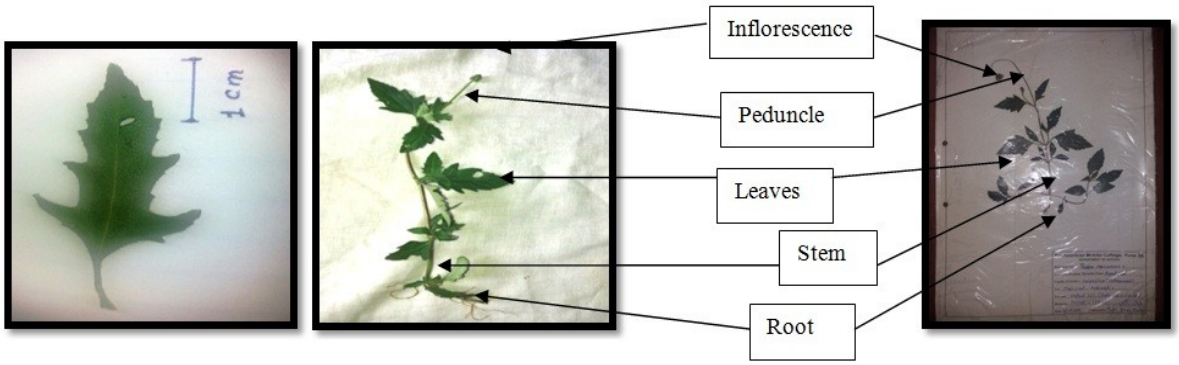


METHODS :

Preparation of plant extract

The fresh leaves were washed thoroughly 2-3 times with running tap water and once with sterile distilled water. The plant leaves were air dried at room temperature and grind to a fine powder using a laboratory grinder. The powder was sieved using 20 mm mesh to obtain a uniform powder for the analysis. Powdered material was maintained at room temperature and protected from light until required for analysis. Extraction was achieved by adding 1 g of powdered material of Methanol. Then the extracts were filtered through filter paper (Whatman no. 1) and filtrate was kept at 4°C temperaturefor further analysis

Determination of total phenol content

Total phenolic content of the extracts were quantified using Folin-Ciocalteu method described by Upadhya et al., (2013)with some modification. The plant extracts (0.125 ml) with distilled water 0.5 ml was mixed with 0.125 ml Folin-Ciocalteu reagent and kept for 10 min for incubation at 37˚C to it 1.25 ml of 7 % sodium carbonate was added and kept for 90 min at room temperature. The absorbance was measured at 760 nm on UV-Visible spectrophotometer. Gallic acid (10–1000 mg/l) was used for calibration of a standard curve and the amount of total phenol was calculated as % dry powder as Gallic acid equivalents (GAE).

Determination of total flavonoid content

Total flavonoid content of all the plant extracts were quantified by using the aluminium chloride colorimetric method describedby Deshmukh et al., (2009). The extracts (1 ml) were mixed with 1 ml 2 % aluminium chloride. The mixture was vortexed and the reaction was kept at the room temperature for 10 min in dark and absorbance of reaction mixture was measured at 367 nm using UV-Visible spectrophotometer. Quercitin (10–200 mg/l) was used for calibration of a standard and the amount of total flavonoid was calculated as mg/100g dry powder as Quercitin equivalents (QUE).

DPPH radical scavenging activity

The antioxidant activities of all the plant extracts were determined by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay described byUpadhya et al., (2015). The DPPH reagent was prepared by dissolving 2.5 mg of DPPH in 100 ml of methanol. The plant extracts (0.1 ml) were allowed to react with 2.9 ml of DPPH reagent. The reaction mixtures were allowed to interact properly and stand in the dark at room temperature for 30 min. The absorbance was measured at 517 nm on UV-Visible spectrophotometer. The percent radical scavenging activity was calculated using following formula:

$$\%RSA = \frac{\text{Abb Blank - Abb treatment}}{\text{Abb Blank}} \times 100$$

Abbreviations:

Total Phenolic content (TPC); Total Flavonoid Content (TFC); DPPH % RSA: Diphenyl Picryl Hydrazyl percent Radical Scavenging Asssay.

RESULTS AND DISCUSSION

Antioxidant capacity

Total phenolic content (TPC) of the methanolic extract was determined by Folin–Ciocalteu method and expressed in mg/mldry powder as Tannic acid equivalents (TAE). It shows 88.02 ± 0.12mg/ml(Table 1). Total flavonoid content (TFC) was determined by $AlCl_3$ method. Results were expressed as mg/100 g dry powder as Quercitin equivalents (QUE). The TFC of *Tridax procumbens* L. was observed to be 47.32 ± 0.87

mg/100 g (Table 2). Antioxidant activity of *Tridax procumbens* L. was also evaluated using DPPH assay. The results show 48.24 ± 0.32 % radical scavenging activity (RSA) (Table 1).

Quantitative estimation by methanolic extract has been recorded interesting finding during Mansoon season 2017. These finding revealed highest concentration of phenolics (88.02 ± 0.12 mg/ml), followed by the methanolic extract assay of *Tridaxprocumbens* L. leaves in 2,2-Diphenyl-1- picrylhydrazyl (DPPH) revealed (48.24 ± 0.32%), and minimum concentration of total flavonoid (47.32 ± 0.87 mg/ml) has been reported, (Table no. 1).

Tridax procumbens L. tnalp is valued for its pharmaceutical properties and medicinal applications.

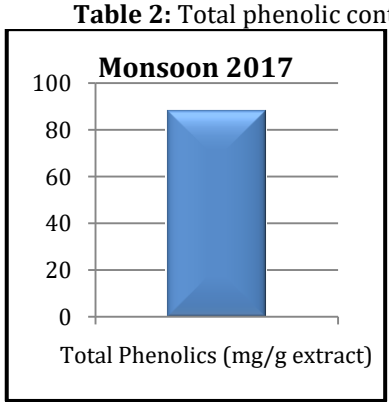
Table no. 1, 2, 3 & 4 shows, Quantitative estimations of total phenolics, total flavonoids and Radical scavenging activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay by methanolic extract (*Tridax procumbens* L. leaves).

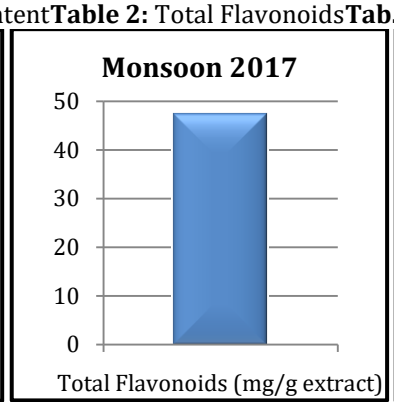
Table 1. Total phenolic content, total flavonoid content and DPPH (RSA)

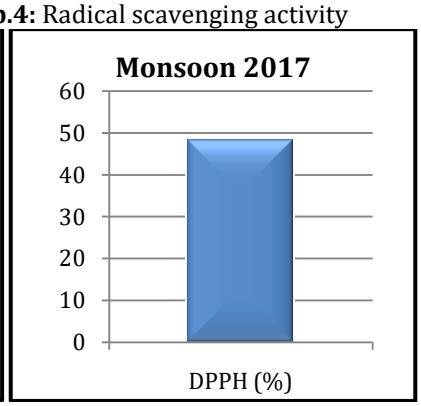
Sample –Rainy Season 2017	Total Phenolics (mg/ml extract)	Total Flavonoids (mg/ml extract)	DPPH (%)
Tridax procumbens L.	88.02 ± 0.12	47.32 ± 0.87	48.24 ± 0.32

Observations from different methods will be depicted in graphical presentation

Table 2: Total phenolic content**Table 2:** Total Flavonoids**Tab.4:** Radical scavenging activity







SUMMARY :

This study of leaves of *Tridax procumbens* L. using methanolic solvents as mentioned in methodology, have been carried separately during these investigations in the mansoon season, which revealed interesting findings valuable for applications in the herbal medicines, ayurvedic medicines, unani medicines and ethanobotany. It may help the people for their health problems. It is also important in the future research in plant physiology. Similarly DPPH of *Tridax procumbens* L. leaves has been presented and quantitative estimation of some bioactive compounds has shown good results.

ACKNOWLEDGEMENT :

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EFFECT OF UVB RADIATION ON NITROGEN METABOLISM IN MEDICINALLY IMPORTANT PLANT SIMAROUBA GLAUCA DC

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ABSTRACT: *The one year old seedlings of medicinally important oil yielding evergreen tree Simarouba glauca were subjected to 10h/day UVB (280-320nm) irradiation treatments for 4, 8,12and16days. While control plants were kept in normal sunlight. The total nitrogen content of root was slightly decreased in 4day and 12days treated plants, while 16 days irradiated shows slight increase in total nitrogen content. In stem tissue there is slight elevation in the total nitrogen content up to 12day after treatment of UV-B irradiations and in leaf tissue it shows inconsistent pattern showing decrease in total nitrogen content in 4, 8 and 16 days after treatments and slight elevation in 12 days treated leaves. The nitrate content in root and stem tissue was decreased with increasing days of UV-B irradiation while in leaf tissue nitrate content was significantly increased with increasing days of UV-B irradiations. The activity of enzyme nitrate reductase was significantly decreased in leaf tissue with increasing treatments of UV-B radiations. This decrease was more significant in 8, 12 and 16 days treated plants. The activity of enzyme Glutamine Synthatase was decreased continuously and this decrease was significant in 8 and 12 days irradiated plants. The free amino acid content of root, stem and leaves was significantly induced due to increased treatments of UV-B irradiations. This increase was more significant in leaf tissue with increasing days of UV-B irradiations. This may helps to improve the energy balance of this plant under UV-B radiation stress. Might be helpful for development of green belts of S. glauca for protection against the UV-B radiations, also helps to reduce green house effects.*

Keywords: *UVB radiation, Nitrate, Nitrate reductase, Glutamine synthatase,Free amino acids etc.*

INTRODUCTION :

The continuously growing population leads to increase the industrial development followed by encouroachment of forest land for industrial set up. This adds various hazardus gases which are responsible for damage of stratospheric ozone layer. The absorption of the single photon of UV by biological molecule leads to the production of exited state to form the higher energy molecule. This will generate reactive oxygen species or free radicals, which can cause several premutagenic damages, cell death. Thus, it is essential to develop the evergreen plant belts to protect the population from these adverse effects of UVB radiations, while simultaneous screening of perennial plant species is need of time to reduce the UV effects on the populations. *Simarouba glauca* is a fast growing evergreen dense foliage tree. It is sturdy and medicinally important oil yielding plant. Medicinal plant *Simarouba glauca* is an evergreen edible oil tree belongs to family Simaroubaceae commonly known as Laxmitaru paradise or dysentery bark.. *S.glauca is* grown in rainfed waste habitat and it is used as ethnobotanical, pharmaceutical and domestic purposes.

A minor component of sunlight is UVB wavelengths (280-320 nm) have a major impact on terrestrial ecosystems because of their high energy levels (Mackenzie, *et al*., 2003 and Caldwell *et al*., 2007). The UV spectrum is divided into three regions UV-C (< 280 nm), UVB and UVA (>320 nm). UV-C radiation that comprises highly energetic wavelengths, which are eliminated by the stratospheric ozone layer and does not reaches on earth surface. UV-B (280-320 nm) reaches on earth surface and encountered by plants and day by day its concentration increases due to the depletion in the stratospheric ozone layer (Caldwell *et al*., 1989).UV-B radiation serves as an environmental stress and triggers several responses in plants,with continuous changes in growth and development, morphological and physiological aspect (Mackerness *et al*.,1998 ; Andrady et.al., 2009 and Hollosy, 2002).It has been estimated that for each 1% sustained decrease in stratospheric ozone, there would be an increase of 0.5% in the number of cataracts caused by solar UV rays(Van der Leun *et al.*1989).

Nitrogen is most prevalent element in living organism with exception of carbon, hydrogen as well as oxygen. Along with many other compounds nitrogen takes part in variable biochemical reactions that composes life of living ones. Thus, this element is a building block of proteins and touchstone of plant productivity.

MATERIALS AND METHODS :

One year old seedlings of *S.glauca* were purchased from social forestry Kagal. Seedlings with plastic bags were kept in playhouse under minimum and maximum air temperature 21 to 31^{0}C respectively and relative humidity of air up to 55%. The seedlings were exposed to UVB radiations artificially supplied by UVB tubes (Philips TL20 W/16NV, Holland). The experiment were carried out at irradiation level 10 h/day for different days such as 4,8,12 and 16 days as per the method described by Lydon *et al.*. (1986). The tubes were suspended perpendicular to the seedlings. and were wrapped with 13 mm cellulose diacetate (CA) film to remove out UVC radiation shorter than 290 nm. CA paper was changed per week to avoid photo degradation. Treatments of UVB radiations were given from 8:00 am to 6:00pm.

The method given by Hawk *et al.,*(1948) is used for determination of total nitrogen content from dry powder of root, stem and leaves. The plant material from without UV-B irradiated control and UV-B irradiated plant material was taken. The Nitrate content was determined by using rapid colorimetric method of Cataldo *et al.* (1975). The activity of enzyme nitrate reductase was determined by *in vivo* method of Jaworski (1971). A method of Lea (1982) was followed for the study of activity of enzyme Glutamine synthatase with modification. The free amino acid contents of the root stem and leaves were estimated following the method of Moore and Stein (1948).

RESULTS :

The effect of UV-B radiation on the activity total nitrogen in *Simarouba glauca* as shown in fig.1.It is noticed from fig. that the total nitrogen content of root is slightly decreased in 4day and 12days treated plants, while 16 days irradiated shows slight increase in total nitrogen content. In stem tissue there is slight elevation in the total nitrogen content up to 12day after treatment of UV-B irradiations and in leaf tissue it shows inconsistent pattern showing decrease in total nitrogen content in 4,12and 16days after treatments and slight elevation in 12 days treated leaves.The overall total nitrogen pattern is somewhat stable in root, stem and leaves of *Simarouba glauca under* UV-B stress.

The effect of UV-B radiation on the nitrate content in root, stem and leaves of *Simarouba glauca* is shown in fig.2. It is observed from fig. that, the nitrate content in root and stem tissue is decreased with increasing days of UV-B irradiation while in leaf tissue nitrate content is significantly increased with increasing days of UV-B irradiations. In the present study nitrate content was increased considerably in leaf tissue and decreased in root and stem tissues. The increased content of nitrate in leaf tissue in response to UV-B radiation is due to the decreased activity of nitrate reductase under UV stress.

The effect of UV-B radiation on the activity of enzyme Nitrate reductase in the leaves of *Simarouba glauca* is shown in fig.3.It is noticed from the fig. that the activity of enzyme nitrate reductase is significantly decreased in leaf tissue with increasing treatments of UV-B radiations. This decrease is more significant in 8, 12 and 16 days treated plants.

The effect of UV-B radiation on the Glutamine Synthatase is as shown in Fig.4. It is observed from fig. that the activity of Glutamine Synthatase is decreased continuously and this decrease is most significant in 8 and 12 days irradiated plants. In the present study, the activity of enzyme Glutamine synthtase was slightly decreased with increasing days of UV-B irradiations.

The effect of UV-B radiation on the Free amino acids in a root, stem and leaves of *Simarouba glauca* is shown in fig.5.It is evident from fig. that the free amino acid content of root, stem and leaves is significantly induced due to increased treatments of UV-B irradiations. This increase is more significant in leaf tissue with increasing days of UV-B irradiations.

CONCLUSION :

There was slight elevation in the total nitrogen content indicating the continuous supply of nitrogen for the development of plants which helps to maintain the inorganic forms of nitrogen in the carbon skeleton and also helps to maintain the synthesis of amino acids such type of role might be contributed by developing this stable nitrogen pool under UV-B radiation stress in *glauca.*

The nitrate content in root and stem tissue was decreased with increasing days of UV-B irradiation while in leaf tissue nitrate content was significantly increased with increasing days of UV-B irradiations. This will affect the nitrate reduction process in leaf tissue as well as the nitrate might be functioning as osmoticum under UV stress in leaf tissue.

The activity of enzyme nitrate reductase was significantly decreased in leaf tissue with increasing treatments of UV-B radiations. This decrease was more significant in 8, 12 and 16 days treated plants.

Further the nitrate reductase is also responsible for the generation of nitric oxide (NO) which acts as reactive gaseous compound and also acts as signaling molecule thus decrease in NR activity will be helpful for the reduction of nitric oxide leading to the reduction in oxidative stress created due to UV-B radiations.

The activity of enzyme Glutamine Synthatase was decreased continuously and this decrease was significant in 8 and 12 days irradiated plants. This may affect assimilation of ammonia.

The free amino acid content of root, stem and leaves was significantly induced due to increased treatments of UV-B irradiations. This increase was more significant in leaf tissue with increasing days of UV-B irradiations. This increased level of free amino acid during the exposure of UV-B irradiation might be due to translocation of free amino acids from source to the sink organs.

This will helps to maintain the various metabolic processes to withstand *S. glauca* seedlings under UVB stress conditions. Further this will helps to maintain such green belts of *S.glauca* seedlings in UVB affected parts of the earth.

Table 1: Effect of UV-B radiation on total nitrogen content of root, stem and leaves of *S. glauca*.

Treatments	Root	Stem	Leaves
Control	1.2	1.1	1.3
4 (Days)	1.13 (-5.8)	1.13 (+2.72)	1.2 (-7.69)
8 (Days)	1.05 (-12.5)	1.34 (+21.81)	1.7 (+30.76)
12 (Days)	1.0 (-16.66)	1.4 (+27.27)	1.28 (-1-53)
16 (Days)	1.4 (+16.66)	0.9 (-18.18)	1.01 (-22.30)

Each value is mean of three determinations.
Values are expressed as g 100^{-1}g dry wt.
Values in parenthesis indicate percent increase (+) or decrease (-) over the control.

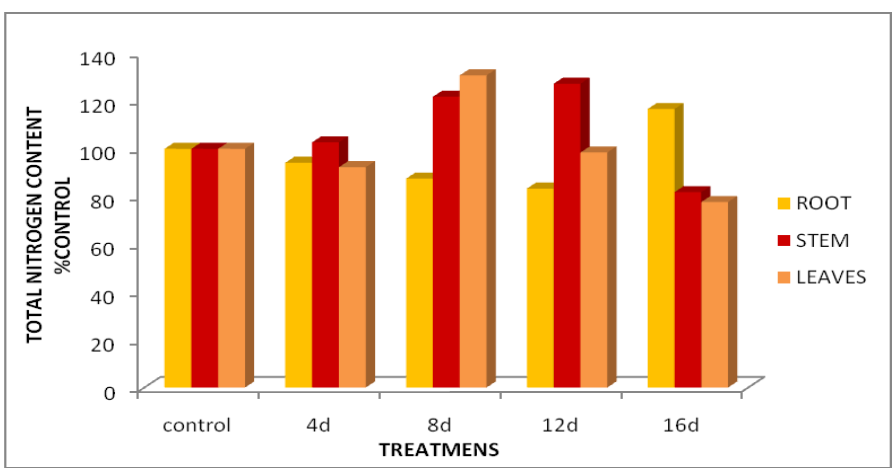

Figure 1 : Effect of UV-B radiation on total nitrogen content of root, stem and leaves of *S. glauca*.

Table 2: Effect of UV-B radiation on nitrate content of root, stem and leaves of *S. glauca*.

Treatments	Root	Stem	Leaves
Control	149	123	158
4 (Days)	103 (-30.87)	103 (-16.26)	185 (+17.08)
8 (Days)	108 (-27.51)	115 (-6.50)	211 (+33.54)
12 (Days)	102 (-31.54)	75 (-39.02)	181 (+14.55)
16 (Days)	109 (-26.84)	68 (-44-71)	240 (+51.89)

Each value is mean of three determinations.
Values are expressed as µg g^{-1} dry wt.
Values in parenthesis indicate percent increase (+) or decrease (-) over the control.

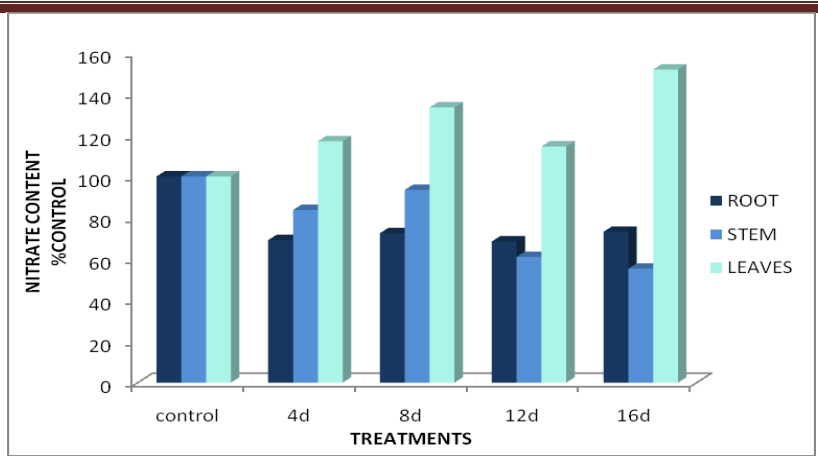

Figure 2: Effect of UV-B radiation on nitrate content of root, stem and leaves of *S. glauca*.

Table 3: Effect of UV-B radiation on the activity of enzyme nitrate reductase in leaves of *S. glauca*.

Treatments	Enzyme nitrate reductase activity
Control	0.41
4 (Days)	0.20 (-50)
8 (Days)	0.129 (-68.53)
12 (Days)	0.142 (-65.36)
16 (Days)	0.12 (-70.73)

Each value is mean of three determinations.
Values are expressed as µmoles of NO_2 lib h^{-1} g^{-1} fresh wt.
Values in parenthesis indicate percent increase (+) or decrease (-) over the control.

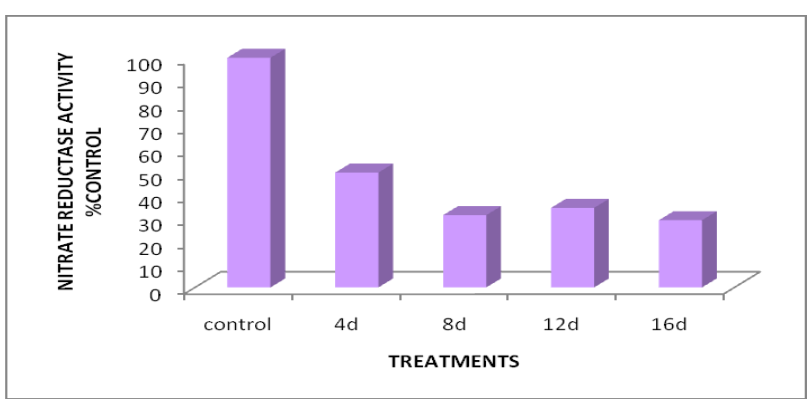

Figure 3: Effect of UV-B radiation on the activity of enzyme nitrate reductase in leaves of *S. glauca*.

Table 4: Effect of UV-B radiation on the activity of enzyme glutamine synthatase (GS) in root, stem and leaves of *S. glauca*.

Treatments	Enzyme glutamine synthatase activity
Control	1.4
4 (Days)	1.38 (-1.42)
8 (Days)	0.8 (-42-85)
12 (Days)	0.52 (-62.85)
16 (Days)	0.96 (-31.42)

Each value is mean of three determinations.
Values are expressed as ΔOD h^{-1} mg^{-1} protein.
Values in parenthesis indicate percent increase (+) or decrease (-) over the control.

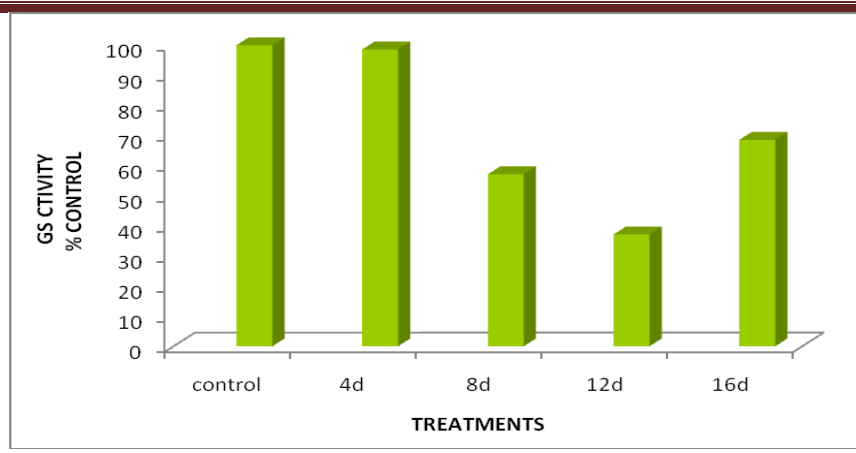

Figure 4: Effect of UV-B radiation on the activity of enzyme glutamine Synthatase (GS) in root, stem and leaves of *S. glauca*.

Table 5: Effect of UV-B radiation on free amino acid content of root, stem and leaves of *S. glauca*.

Treatments	Root	Stem	Leaves
Control	214	257	321
4 (Days)	257 (+20.09)	314 (+22.17)	350 (+9.03)
8 (Days)	450 (+110.28)	111 (-56.80)	664 (+106.85)
12 (Days)	814 (+280.37)	814 (+216.73)	771 (+140.18)
16 (Days)	471 (+120.09)	385 (+49.80)	750 (+133.64)

Each value is mean of three determinations.
Values are expressed as mg 100^{-1}g dry wt.
Values in parenthesis indicate percent increase (+) or decrease (-) over the control.

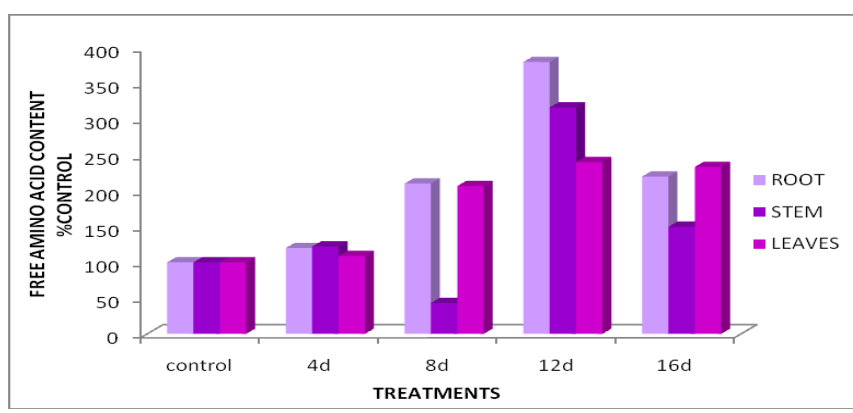

Figure 5: Effect of UV-B radiation on free amino acid content of root, stem and leaves of *S. glauca*.

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TO STUDY EFFECT OF SUBSTRATE ON MORPHOLOGY AND PHOTOELECTROCHEMICAL PERFORMANCE OF CuS-CdS HETEROJUNCTION FOR SOLAR CELL APPLICATION

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ABSTRACT: *The CdS-CuS heterojunction films have been deposited on the FTO and on the copper substrates to study the effect of Substrate on morphology and Photoelectrochemical performance of the cell. The films are characterised using X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and compared the photoelectrochemical performance for the solar application.*
XRD patterns of CuS-CdS indicates peaks corresponding to CuS (hexagonal) and CdS (cubic) phases. SEM images of CdS-CuS heterojunction on FTO shows tetrapod while on the copper substrate it gives compact rod-like morphology which ultimately affects the cell performance. Study the Photo-electrochemical performance by using a conventional two-electrode system which reveals that device with copper substrate gives better conversion efficiency (1.4 %) as compared to the FTO substrate (0.11 %).

Keywords: *Sol-gel, Photocatalysis, Nanocomposite.*

I. Introduction

The demand of energy tremendously increasing with progression in technology which is creating stress on non-renewable sources of energy, as the non-renewable energy resources remain limited need to focus on renewable resources which can replace these sources, among renewable resources solar energy is the best alternative for conventional sources. Using a solar cell device can convert light energy from the sun into electrical energy. Verity of the semiconducting material is available for a solar cell device [1]. Heterostructures of the semiconductor are advantageous as containing two or more components, which creates multifunctional materials [2] for fabrication of heterojunction variety of material combinations with a methodology of successive growth are available, but it's difficult to separate nanocrystals of the secondary material often enters with homogeneous nucleation with the desired heterogeneous nucleation on the nanocrystal surface [3]. In ionic nanocrystals, the composition of the material changed by using cation exchange reactions by replacing the cations within the nanocrystal lattice with the different metal ion [5, 6]. The nanostructured heterojunction is the important topic of a wide area of research for many optoelectronic devices such as semiconductors, LASER, photodetectors, solar cells, etc. [7, 8, 9].

The choice of material important as it affects the performance as well as the economy of the device. The reduction of the cost of the thin film cells achieved by minimizing the utilization of materials, inexpensive material processing method [10]. Due to various stoichiometric compositions, unique structures, and wide area of applications Copper sulfide fascinate considerable attention [11, 12]. CdS due to it's wide and direct band gap transition (2.42 eV) used in solar cells [13]. Using a combination of these two semiconductors fabricate CdS-Cu_2S heterojunction junction solar is which is the low-cost device for direct conversion of solar radiation into electricity. Recently the cells have expanded interest as a possible means of large-scale terrestrial power generation [14, 15].

In our previous work, we reported the electrolytes effect of Cu_2S–CdS heterojunction on a copper substrate. Current article focuson a comparative study of substrate effect using copper and FTO the deposition of heterojunctions carried out by CBD and chemical method.

II. Experimental procedures:

2.1. On the glass substrate

A) Deposition of CuS

The CuS film was deposited on FTO substrate (150 nm on glass) via CBD method before the deposition FTO substrate were cleaned ultrasonically in acetone for 10 min washed by deionised water. 10 ml of 0.1M $CuSO_4$ solution taken in reaction beaker to this added 5 ml of TEA stirred for 2 min to make homogenous solution followed by addition of 20 ml ammonia dropwise and maintain pH of solution 11. The total volume of the reaction bath was made 200 ml by adding DI stirred solution add 10 ml of thiourea. Precleaned FTO glass substrate was immersed vertically in a reaction bath and then kept at 70°c with

constant stirring 50 rpm after ½ an hour get CuS thin film [16].

B) Fabrication of the CuS/CdS heterojunction:

CdS thin films synthesized onto previously deposited CuS film by using simple chemical bath depositions (CBD) technique with deposition temperature maintained at 35°- 40° C using cadmium sulphate ($CdSO_4$) and thiourea ($SC(NH_2)_2$) as a source of CdS and S respectively. During deposition, precursors prepared by mixing cadmium sulphate (5 ml, 0.1 M), ammonia (~40 ml, 28 %), 50 ml deionised water and thiourea sequentially (5 ml, 0.1 M). The pH of the bath is adjusted to ~12 by addition of sufficient quantity ammonia. The CuS thin film was deep in a bath. The deposition temperature was kept constant at 323 K, after 1 hr to obtain yellowish-orange CdS thin film was obtained.

2.2. On the copper substrate

Polysulphide used as a source of sulphur the copper substrate dipped in 0.1 M polysulphide solution for a couple of minutes. The obtained black layer of CuS thin film washed with deionised water dried naturally and stored in a desiccator. CuS film annealed at 373 K for 1 hr. Fabrication of CuS-CdS heterojunction was carried by deposited of CdS on CuS thin film by CBD using same bath composition and reaction condition which used for the glass substrate.

2.3 Characterisation technique :

The films characterised using a D/MAX Uitima III XRD spectrometer (Rigaku, Japan) with CuKα line of 1.5410 Å for 2θ in 10 to 90° ranges. The study of surface morphology was carried out using a scanning electron microscope (SEM) (The JEOL JSM-6360). Photoelectrochemical properties compared using two electrodes systems, a graphite rod as a counter electrode and a CdS-CuS thin film as a working electrode (10 mW/cm^2 illumination of a Tungsten lamp)

III. Results and discussion

3.1 X-ray Diffraction Analysis

The phase purity with element confirmation of material obtained material studied by using X-Ray Diffraction pattern.

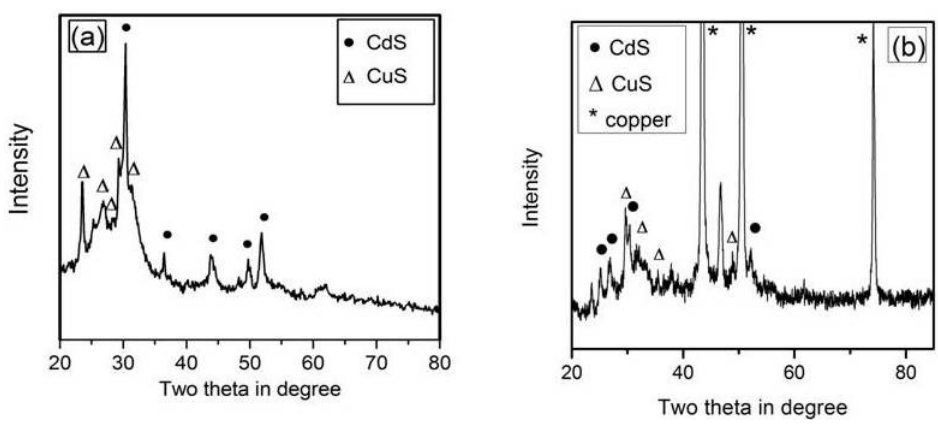

Fig. 1: XRD diffraction pattern of CuS-CdS thin films (a) on a glass substrate (b) on a copper substrate

Fig 1a shows the XRD patterns of CuS-CdS heterojunction on the glass substrate. The diffraction pattern shows peaks at 2θ = 26.61, 36.52, 51.81° and 30.58, 43.99 ° are index for the planes of Cubic (111), (002), (112), and Hexagonal (200), (220) structure of CdS in accordance to JCPDS Card (No.75-1546) and (41-1049) respectively, characteristic peak of CuS Hexagonal at 2θ 29.27, 31.58° corresponds to plane (102), (103) are in good agreement with JCPDS Card (No. 08-0464). Fig 1b shows XRD pattern of the heterojunction on the copper substrate the peaks due to CdS are intense and identified as the reflections of 26.58, 30.65, 50.9° due to planes (111), (200), and (311) respectively match with JCPDS Card (No.75-1546). The diffraction peaks at 29.5, 31.78° are assigned to the plane (102), (103) of the hexagonal CuS phase; these peaks are good agreement with a standard data card (No 75-2233). The crystallite size calculated using Debye-Scherer's formula [17].

$$D = \frac{(0.9 \times \lambda)}{(\beta \cos\theta)} \quad \text{.....(1)}$$

Where 'D' is the crystallite size, λ the X-ray wavelength used, 'β' full width of half maximum intensity and 'θ' the Bragg's diffraction angle, observed. The concentration of precursor controls the crystallite size which

increases with the increases concentration of precursor [18]. The chemical method used for deposition of CuS on a copper substrate of sulphide precursor concentration is high as compared sulphide precursor used in CBD for deposition of CdS on a glass substrate as a results crystallite size of CuS 67.43 nm on a copper substrate which is greater than on a glass substrate 44.08 nm. In case of CdS Crystallite size approximately same size on glass and copper substrate 30.06 and 30.80 nm respectively due to the same solution of the equal concentrations used for deposition followed by the same method.

3.2 Surface Morphology in solar energy application

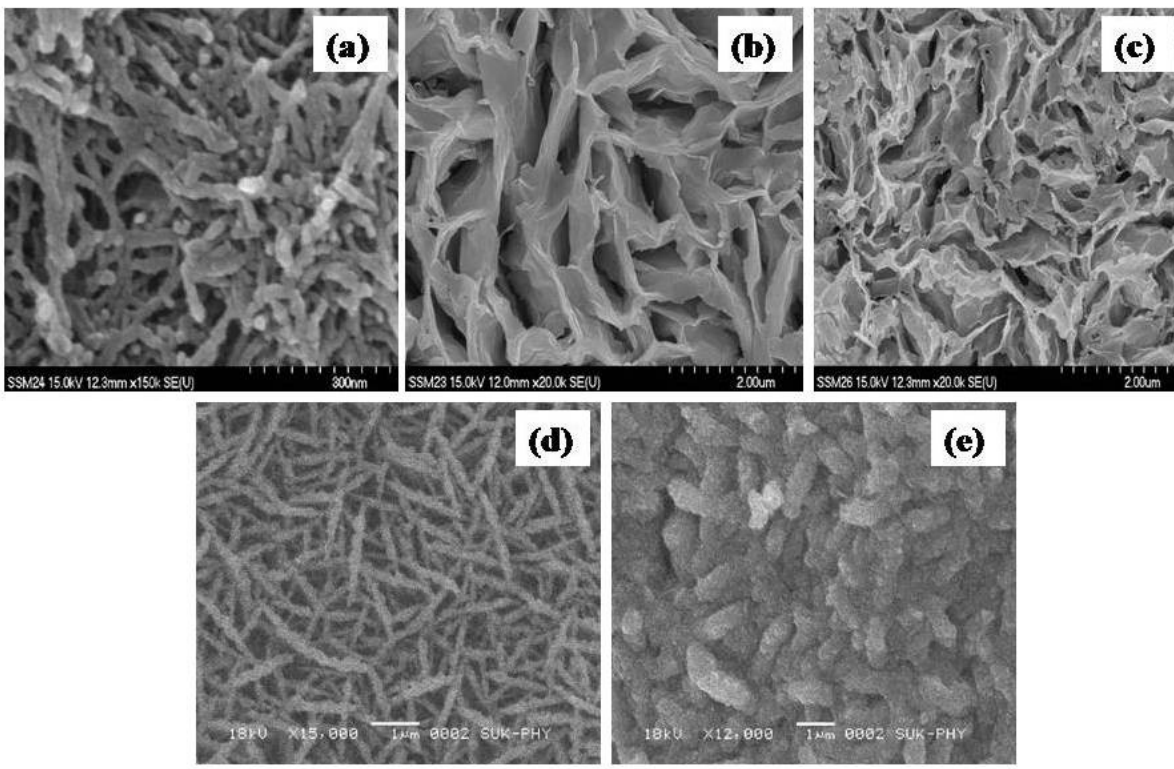

Fig. 2: SEM images of (a) CuS, (b) CdS and (c) CuS-CdS heterojunction thin film on glass substrate

Surface morphology of material plays an important role in the solar energy application. Fig 2a & 2b show the morphology of CuS & CdS respectively on the glass substrate, CuS form an internet of the nanowire of diameter near about 32 nm, while CdS form vertically aligned flakes which form an adherent porous sheet of a tetrapod. SEM of heterojunction shows adherent compact tetrapod. The initial morphology of CuS film does not retain after CdS deposition on the glass substrate as shown in fig 1c. The CuS on the copper substrate forms a rod of thickness about 190 nm with a length of approximately 2.5 μm. The growth of CdS over CuS rod which results in increases compactness & thickness of rod up to 500 nm as shown in fig 2d and 2e. In the case of the copper substrate, it observed that the ion lattice is conserved this allows retaining the initial morphology of CuS to preserved after the junction formation [19, 20]

3.3 Photoelectrochemical Performance

The combination of a p-n junction with a Schottky barrier to producing a combined p-n junction-Schottky barrier or it is also called a "hybrid" diode (21). Fig 3 shows a schematic diagram of working of CuS-CdS heterojunction.

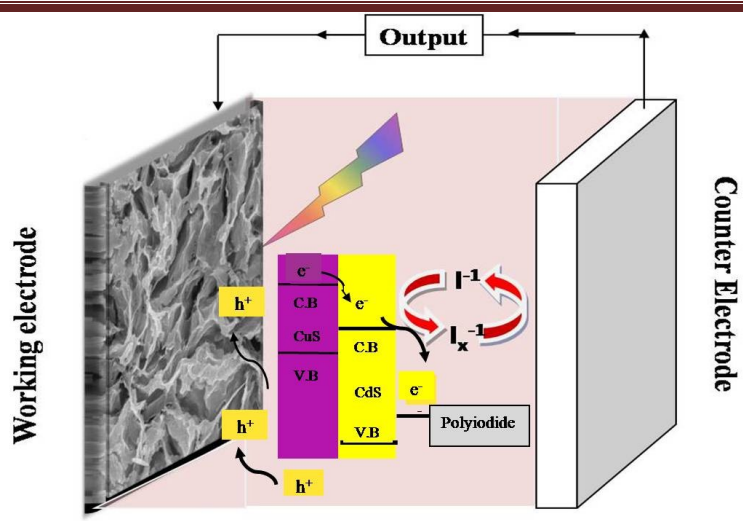

Fig. 3: Schematic diagram of working of CuS-CdS heterojunction.

The photoelectrochemical performance of CuS-CdS thin film using different substrate was investigated using a standard two electrode configuration at an illumination of 10 mW/cm^2. The conversion efficiency was calculated using relation (3).

$$\eta\,(\%) = \frac{P\max}{Pin} \times 100 \qquad \text{.....(3)}$$

Where P_{max} is the maximum output power registered for a cell and Pin the input power. P_{max} was calculated from the power output curve and given by $P_{max} = V_{max}\, I_{max}$. The fill factor (FF) was calculated using equation (6).

$$FF = \frac{Vm\,ax \times Imax}{Voc \times Isc} \qquad \text{.....(4)}$$

The conversion efficiency of PEC Cell has been obtaining from power output characteristics. Values of efficiency and fill factor are found to be 1.43 % and 0.45 respectively for the copper substrate and on the glass; it is 0.11 % and 0.37 respectively. Use of the copper substrate provides additional Schottky junction to the p-n junction increase in efficiency of the cell due to the advantage of the high current capability of the p-n junction together with the switching properties and the low forward voltage drop of the Schottky junction (22).

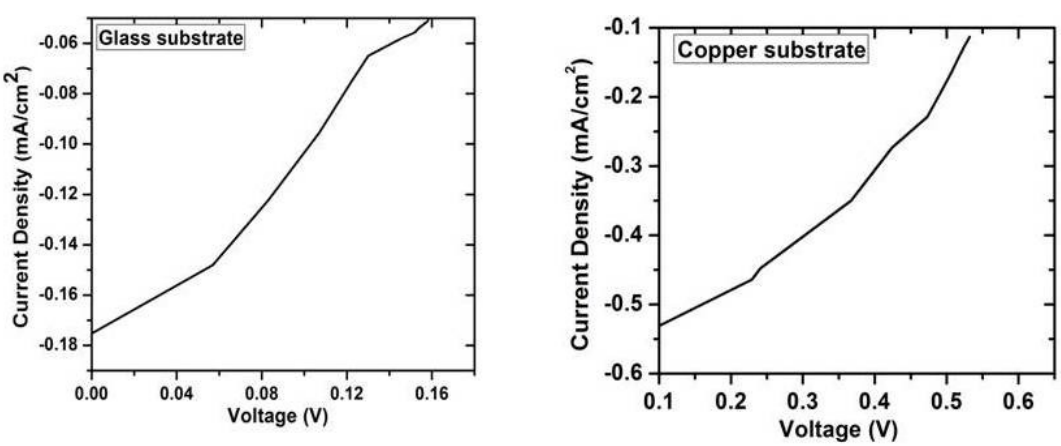

Fig. 4: Photoelectrochemical performance of CuS-CdS heterojunction (a) on a glass substrate (b) on copper

IV. Conclusion

It has been observed the substrate plays a decisive role in the efficiency of the PEC cells. The performance of the p-n junction solar cell possible improved by fabrication of Schottky junction in addition to the p-n junction for CuS-CdS heterojunction efficiency on FTO is 0.11% which increased efficiently on copper substrate & it reach up to 1.43%.

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Facile Knoevenagel Reaction using Gel Entrapped Base Catalyst for the synthesis of pyrano[3,2-c]pyran and pyrano[3,2-c]chromene scaffolds

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ABSTRACT: *A simple, efficient and green route for the synthesis of pyrano[3,2-c]pyran and pyrano[3,2-c]chromene scaffolds has been developed by multicomponent condensation of mixture of aldehydes, malononitrile and 4-hydroxy-6-methyl-2H-pyran-2-one/4-hydroxy coumarin in the presence of KOH-Gel entrapped base catalyst affording good to excellent yields under neat conditions. The developed protocol is environmentally benign which avoids toxic solvents, chromatographic separation and use of expensive catalysts.*

Keywords: *pyrano[3,2-c]pyran, pyrano[3,2-c]chromene, KOH-Gel entrapped base catalyst*

I. Introduction

The traditional multistep chemical synthesis generally includes a number of synthetic operations, such as extraction, filtration, purification and crystallization. Multicomponent reactions (MCRs) allow remarkable advantages like, ease of operation, simplistic automation, reduce the steps which involve extraction or purification processes and follow the eco-friendly transformations [1]. One-pot MCRs often reduce the reaction time and provide higher chemical yield comparative to multiple-step synthesis, which automatically trims down the use of energy and manpower. MCRs are very much constructive in the convenient formation of diverse organic molecule having pharmaceutical as well as industrial applications [2]. Therefore, the synthesis of heterocyclic organic compound via MCRs has attracted great attention, especially in the areas of organic synthesis.

4*H*-Pyrans and 4*H*-pyran-annulated heterocyclic skeleton is an "esteemed" structural pattern [3] thoroughly distributed in significant biological active molecules [4] such as antitumor [5], vasodilatatory agent [6], anti-cancer [7], anti-HIV [8], anticoagulant [9], anti-AIDS [10], potent monoamine oxidase (MAO) inhibitors [11], antileishmanial and antiviral agents [12,13]. Due to broad biological active spectrum, novel techniques and versatile catalysts were reported for the synthesis of 2-amino-4*H*-pyran and 2-amino-4*H*chromene derivatives, such as grinding [14], ionic liquid [15], microwave [16], ultrasound [17], IR radiation [18], reflux using organic solvents [19], organic catalyst [20], synthetic surfactants [21] and phase transfer catalyst [22].

Although the literature on 4*H*-Pyrans and 4*H*-pyran-annulated heterocyclic skeleton contains a rich array of versatile methodologies, new approaches remain valuable additions to the contemporary arsenal of synthetic strategies. The concept of Gel-Entrapped Base Catalysts (GEBCs) combines the advantages of alkali and organic bases with those of heterogeneous supports [23]. These catalysts are prepared by immobilization of alkali or organic bases by entrapping them in an aqueous gel matrix of agar-agar which reduces the amount of bases used, and allows easy and efficient separation of products from the catalyst. Besides this, bases like alkalis, when exposed to air, absorb moisture and are spoiled. On the contrary, the GEBCs do not absorb moisture on exposure to air and remain intact. This also provides an excellent opportunity of recyclability and reusability, which are seldom possible using bases alone as catalysts. The interesting properties of GEBCs spurred us to probe their barely exploited potential in organic synthesis. In our continuing search for novel catalysts in organic synthesis [24], we report herein the synthesis of pyrano[3,2-c]pyran and pyrano[3,2-c]chromene scaffolds by multicomponent condensation of mixture of aldehydes, malononitrile and 4-hydroxy-6-methyl-2*H*-pyran-2-one/4-hydroxy coumarin in the presence of KOH-GEBC affording good to excellent yields under neat conditions.

II. Results and Discussion

2.1 Preparation of Gel Entrapped Base Catalysts & characterization (GEBCs):

To a boiling mixture of agar-agar (20 mL) in water (60 mL) was added a mixture of base KOH (10 gm) in water (10 mL). The resultant solution was boiled with stirring for five minutes and cooled in ice bath to yield the desired KOH-GEBC. The GEBCs were light yellow jelly like substances that could be cut into small cubic shape pieces **(Fig. 1a).** The same procedure is used for preparation of piperidine-GEBC and Morpholine-GEBC NaOH-GEBC. The nature and appearance of these GEBCs is just like as that of KOH-GEBC.

Fig. 1a Photograph of KOH GEBC

Fig.1b Photograph of Morpholine-GEBC

2.2 Characterization of Gel Entrapped Catalysts (GECs): TGA-DTA analysis

Thermal behaviour of GEBCs was studied by thermogravimetric analysis (TGA) and differential thermal analysis (DTA). The TGA curves of KOH-GEBC **(Fig. 2a)** revealed that degradation of gel occurs with increase in temperature and completes at 151°C as evident from strong endothermic peak observed in DTA. The thermal decomposition of agar polymer starts around 240°C and is very slow up to 475°C indicating that the polymer matrix may undergo some structural changes leading to complete exothermic decomposition of polymer material around 500°C leaving only anhydrous KOH which decomposes above 800 °C.

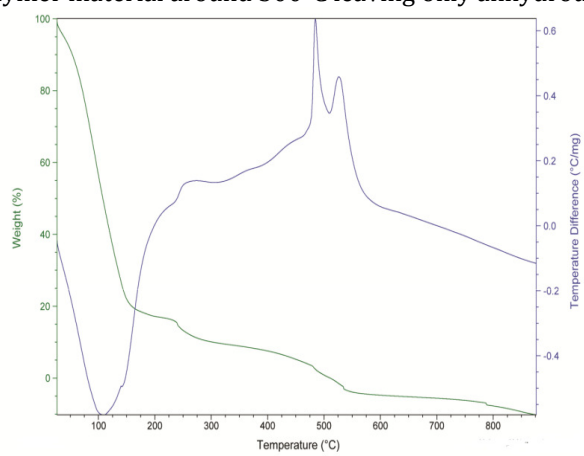


Fig. 2a: TGA-DTA of KOH GEBC

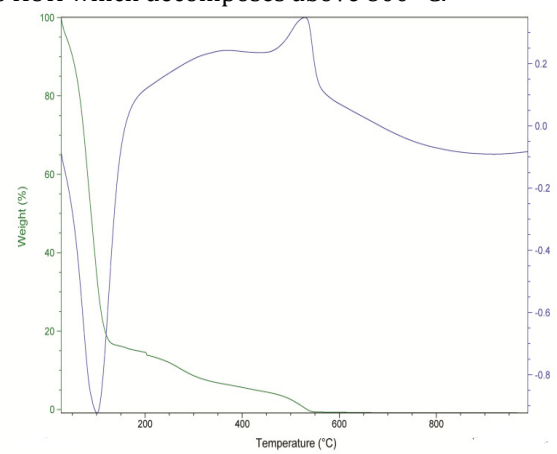


Fig. 2b:TGA-DTA of Morpholine GEBC

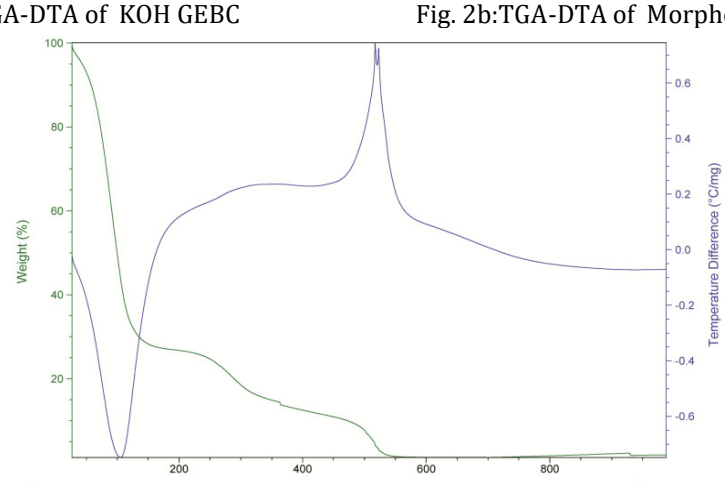


Fig.2c: TGA-DTA of Piperidine GEBC

However, in the case of GEBCs containing morpholine and piperidine**(Fig. 2b** and **2c)**, the decomposition of bases is observed before the degradation of polymer. The process of base degradation is slow due to intercalation of morpholine and piperidine in polymer matrix.

A model reaction was carried out using equimolar mixture of benzaldehyde (**1a**), malononitrile (**2**) and 4-hydroxy-6-methyl-2*H*-pyran-2-one (**3**) was taken which was stirred in the presence of 1 gm of various GEBCs in ethanol at ambient temperature till the completion of reaction as monitored by thin layer chromatography. The results are summarized in **(Table 3.1)**. Amongst the various GEBCs, KOH-GEBC was found to be better than piperidine and morpholine GEBCs.

Table 1: Screening of various GEBCs for synthesis of pyrano[3,2-c]pyran and pyrano[3,2-c]chromene [a]

Entry	GEBC	Time (Min)	Yield (%)
1	**KOH-GEBC**	**25**	**94**
2	Morpholine-GEBC	25	68
3	Piperidine-GEBC	25	75

[a]**Reactionconditions:**benzaldehyde (**1a**), malononitrile (**2**) and 4-hydroxy-6-methyl-2*H*-pyran-2-one (**3**)
[b]**Isolated yields.**

The stupendous increase in the yield of product as well as reaction time of KOH-GEBC may be attributed to higher basicity of KOH as compared to piperidine and morpholine. The effect of solvents on a model reaction using KOH-GEBC was studied. It was observed that in solvents like 2-propanol, methanol, dichloromethane and toluene, the yields of the product were considerably lower than that in ethanol **(Table 3.2)**. It is worthy of note that in blank experiment no reaction was observed under similar conditions in the absence of GEBC. The striking feature of all the reactions was the isolation of products. It was interesting to observe that after specified time, the product precipitates out of the reaction mixture that can be isolated simply by filtration. The product obtained after sufficient washing with water was found to be practically pure.

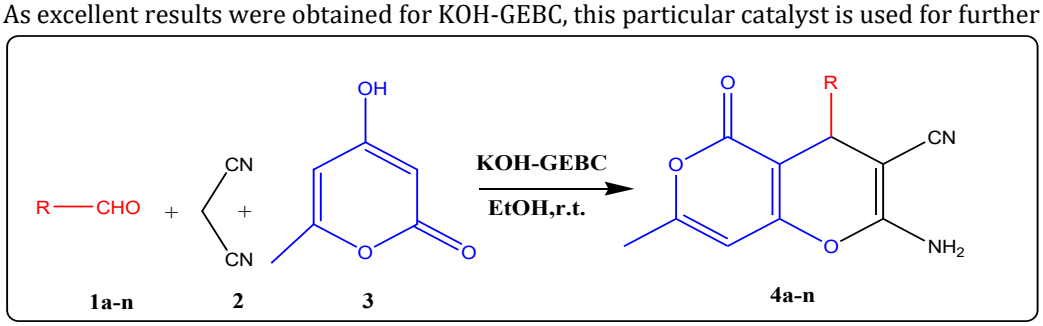
Table 2: Solvent effect on the synthesis of pyrano[*3,2-c*]pyranand pyrano[*3,2-c*]chromeneusing **KOH-GEBC**[a]

Entry	Solvent	Time (Min)	Yield (%)
1	**Ethanol**	**25**	**94**
2	2-Propanol	25	75
3	Methanol	25	65
4	Dichloromethane	25	52
5	Toluene	25	42

[a]**Reactionconditions:**benzaldehyde (**1a**), malononitrile (**2**) and 4-hydroxy-6-methyl-2*H*-pyran-2-one (**3**)
[b]**Isolated yields.**

After completion of the reaction, the KOH-GEBC was separated from reaction mixture, washed with ethanol and reused in another reaction with identical substrates. The catalyst showed a remarkable recyclability as the yield of the product decreased slightly from the first run to fifth run.

As excellent results were obtained for KOH-GEBC, this particular catalyst is used for further studies.



Scheme 1

To investigate the feasibility of KOH-GEBC, a number of structurally diverse benzaldehyde (**1a**), malononitrile (**2**) and 4-hydroxy-6-methyl-2*H*-pyran-2-one (**3**) were reacted in ethanol **(Scheme 1)**. The results are summarized in **(Table 3)**.

Table 3: Synthesis of pyrano[*3,2-c*]pyranderivatives.[a]

Sr. No.	R	Product	Time (min)	Yield[b](%)	M. P.°C
1	C_6H_5	4a	20	90	236-237 [25]
2	4-F-C_6H_5	4b	15	92	220-222 [25]
3	2-Cl-C_6H_5	4c	20	90	268-270 [27]
4	4-Cl-C_6H_5	4d	20	90	230-231[27]
5	2,4-di-Cl-C_6H_5	4e	15	90	234-235 [27]
6	3-Br-C_6H_5	4f	20	93	218-220 [25]
7	4-Br-C_6H_5	4g	20	92	230-232[25]
8	3-NO_2-C_6H_5	4h	20	92	239-241[25]

9	4-NO_2-C_6H_5	4i	20	90	218-220 [25]
10	4-CN-C_6H_5	4j	20	88	219-220 [26]
11	4-CH_3-C_6H_5	4k	20	91	217-219 [25]
12	3,4-di-OMe-C_6H_5	4l	20	90	199-200 [25]
13	Furfural	4m	20	89	119-120 [25]
14	Thiophene-2-aldehyde	4n	20	90	239-240 [25]

[a]**Reaction conditions:** aldehyde (1 mmol), malononitrile (1 mmol) and 4-Hydroxy-6-methyl-2*H*-pyran-2-one (1 mmol) in 5 mL Ethanol added 1 gmKOH-GEBC stirred at R.T at room temperature.
[b]**Isolated yield of the product**.

III. Experimental Section

3.1Materials and Methods

The reaction was monitored by thin layer chromatography using 0.25 mm Merck silica gel 60 F254 precoated plates, which were visualized under UV light. Melting points were determined in an open capillary and are uncorrected. Infrared spectra were recorded on Perkin Elmer FT-IR spectrometer (KBr discs ~5% *w/w*). NMR spectra were recorded on Bruker Avon 300 MHz and 75 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal reference.

3.2 General procedure for synthesis of pyrano[3,2-c]pyran derivative / pyrano[3,2-c]chromene

A mixture of aldehyde (1 mmol), malononitrile (1 mmol) and 4-Hydroxy-6-methyl-2*H*-pyran-2-one (1 mmol) / 4-hydroxycoumarin (1 mmol)was stirred in the presence of GEBC (1 gm) in 5 mL of ethanol at ambient temperature till the completion of the reaction as monitored by TLC. The resulting crude product

was filtered off, washed with ethanol and recrystalized from same solvent (ethanol) to afford pure products. The purified compounds were characterized by various spectral analyses [IR, ^{1}H NMR, ^{13}C NMR , MS].

IV. Conclusion

In summary, we have developed a novel and highly efficient methodology for MCR one pot synthesis of aldehydes (1 mmol), malononitrile (1 mmol) and 4-Hydroxy-6-methyl-2H-pyran-2-one/ 4-hydroxycoumarin (1 mmol) compounds using recyclable KOH-GEBC. Furthermore, this method is of interest in the context of green chemistry. The method offers several significant advantages, such as high conversions, easy handling, clean reaction profile, atom economy, air-stable, moisture insensitive and short reaction time, which make it an useful and attractive addition to the existing methodologies for the synthesis of MCR products.

V. Spectral Data:

2-Amino-3-cyano-4-(4-nitrophenyl)-4*H*, 5*H*-pyrano[*3,2-c*]pyran-5-one (Table 3, Entry 9, product 4i):IR (KBr) *ν* 3302, 3178, 2201, 1698, 1515, 1344, 1262, 862, 742 cm^{-1} , **1H NMR (300 MHz, DMSO-d_6)**: 2.20 (s, 3H, CH_3), 4.45 (s, 1H, chiral CH), 6.13 (s, 1H, =CH), 7.12 (s, 2H,NH_2), 7.44 (d, 2H, *J*= 8.4 Hz, Ar-H), 8.12 (d, 2H, *J*= 8.7 Hz, Ar-H) ppm, **^{13}CNMR (75MHz, DMSO-d_6)**δ: 19.9 (CH_3), 36.6 (chiral CH), 57.2 (carbon attached with CN), 98.5, 100.1, 123.7, 129.1 (CN), 146.9, 150.7, 158.7, 159.0, 161.9 and 163.3 (carbonyl carbon of lactone) ppm.

2-Amino-4-(4-fluorophenyl)-3-cyano-4*H*, 5*H*-pyrano[*3,2-c*]chromene-5-one (Table 3, Entry 2, product 4b: IR (KBr) *ν*: 3369, 3334, 2195, 1714, 1505, 1348, 1054, 765 cm^{-1}, **1H NMR (300 MHz, DMSO-d_6)**δ: 4.61 (s, 1H, chiral CH), 7.07 (bs, 2H, NH_2), 7.27 (d, 1H, *J*= 8.4 Hz, Ar-H), 7.34 (t, 1H, *J*=7.8 Hz, Ar-H), 7.46 (d, 2H, *J*= 8.4 Hz, Ar-H), 7.58 (t, 1H, *J*= 8.1 Hz, Ar-H), 7.90 (d, 1H, *J*=7.8 Hz, Ar-H), 8.09 (d, 2H, *J*= 8.4 Hz, Ar-H) ppm, **^{13}C NMR (75MHz, DMSO-d_6)**δ: 37.3 (chiral CH), 57.1 (carbon attached with CN), 103.1, 113.1, 116.8, 119.1, 123.2, 123.9,125.0, 133.4, 147.0, 150.8, 152.6, 154.4, 158.6 and 160.0 (carbonyl carbon of lactone) ppm.

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Tribulus terrestris aqueous leaves extract mediated synthesis of copper nanoparticles and its characterization

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ABSTRACT: *A facile and green route for the synthesis of amorphous nature copper nanoparticles (CuNPs) was developed by using renewable natural resource Tribulus terrestris aqueous leaves extract as the reducing agent. The structural investigation of biosynthesized CuNPs was performed using ultraviolet-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction study (XRD) and selected area electron diffraction (SAED) analysis. The amorphous nature of the CuNPs was identified using XRD and SAED analysis, whereas average nanoparticle size of CuNPs was ~69 nm shown by TEM analysis.*

Keywords: *amorphous copper nanoparticles, bioreduction, Tribulus terrestris*

I. Introduction

Copper is one of the valuable soft metal which occur in the nature in elemental as well as in ores of oxide, sulphide, form. It is used in variety of applications such as, building materials, decorative art, house hold utensils, in electric wires, etc. Copper is essential to all living organisms because it is a key constituent of respiratory enzyme complex cytochrome-c oxidase. In some organisms it acts as blood pigment hemocyanin where the iron-complexed hemoglobin replaced by copper-complex. Like bulk, nanoparticulate form of copper has wide range of applications in industrial as well as medical field such as biocidal, antibacterial, sensors, catalytic process, high temperature superconductors and solar cells [1-6]. Due to wide range of applications of CuNPs, scientists have attracted much attention for its synthesis.

In literature, production of CuNPs achieved through different methods. Chemical methods are the most popular methods for the synthesis of CuNPs such as radiolysis reduction, thermal decomposition, vapor deposition, chemical reduction, electrochemical reduction, [7-11] etc. However, some chemical methods include use of toxic chemicals in their synthetic protocol, expensive reagents, reaction carrying out at high temperatures and pressures. Since metal nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of CuNPs synthesis that has to avoid toxic chemicals. Biological methods like by using microorganisms or plant extract [12-13] have been suggested as possible ecofriendly alternatives to chemical and physical methods.

Although the literature on the synthesis of CuNPs contains a rich array of versatile methodologies, new approaches remain valuable additions to the contemporary arsenal of synthetic strategies. So, the present paper reports a facile and green route for the synthesis of amorphous nature copper nanoparticles (CuNPs) by using renewable natural resource *Tribulus terrestris* aqueous leaves extract as the reducing agent at room temperature. *Tribulus terrestris* is an annual plant, native to warm temperate and tropical regions widely distributed around the world comes under Zygophyllaceae family.

II. Materials and Methods

2.1 Materials

All the chemicals used were of analytical grade and obtained from Molychem. Fresh, green and mature leaves of *Tribulus terrestris* (**Fig.1)** plant were collected from "University campus, Shivaji University, Kolhapur"(MS, India) and the taxonomic identification was made in Department of Botany, Shivaji University, Kolhapur (MS, India). All solutions were freshly prepared using double distilled water and kept in dark at room temperature for reduction of copper ions.

Fig.1: ***Tribulus terrestris*** **plant**

2.2 Preparation of Aqueous Extract of Tribulus terrestris Leaves

Fresh, green clean leaves of *Tribulus terrestris* were air dried and kept in the hot air oven at 60 °C for 24 h. The dried leaves were ground to a fine powder and 2 g of powder was taken in 500 mL Erlenmeyer flask containing 50 mL double distilled water. The resulting mixture was then boiled for 30 minutes and filtered through Whatman filter paper no. 41. This concentrated aqueous extract (30-35 mL) was kept in refrigerator for further use.

2.3 General procedure for AgNPs synthesis

For the synthesis of CuNPs, 5 mL aqueous extract of *Tribulus terrestris* leaves was taken and diluted to 25 mL by double distilled water. This diluted extract then added drop wise in 25 mL of silver nitrate solution (1 mM) at room temperature with constant stirring for 5 hours. The resulting brown solution was centrifuged at 14,000 rpm for 20 minutes and then washed with distilled water (3x10 mL). The solid obtained was dried under vacuum and used for further study

2.4 Characterization Methods and Instruments

Synthesized CuNPs were characterized by UV-visible spectroscopy, X-ray diffraction (XRD), Selective area electron diffraction study (SAED), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). The UV-visible spectra were recorded over 200-800 nm range with UV-3600 PC UV-VIS NIR Spectrophotometer (Shimadzu). XRD patterns were recorded on Bruker AXS model D-8, (10 to 70°range, scan rate = 1° min-1) equipped with a monochromator and Ni-filtered Cu Kα radiation. SEM was performed using a HITACHI S-4800 instrument to study the morphology of CuNPs. The TEM analysis was performed on a Jeol model JEM 1200 electron microscope operated at an accelerating voltage of 120 kV.

III. Result and Discussion

The complete reduction of Cu^{2+} ions was visually observed by change in color from blue to brown after the drop wise addition of aqueous extract of *Tribulus terrestris* leaves and then confirmed by UV-Visible spectroscopy. The color change observed due to the surface plasmon vibration in the CuNPs and the absorbance maxima appeared at 270 nm after 2.5 hours in UV region **(Fig 2)**.

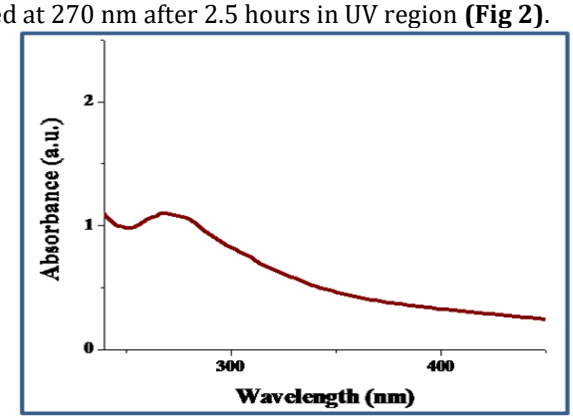

Fig.2: UV-Visible spectra of biosynthesized CuNPs

The **Fig. 3a** showed XRD pattern of the biosynthesized CuNPs which is dense descending slope with no sharp peaks and not shown sharp diffraction peaks related to extended crystalline structure. Instead, a broad band is seen to appear which is typical for amorphous materials and for ultra-small crystalline

materials where diffraction peaks cannot be well resolved. The selected area electron diffraction (SAED) also demonstrates the less crystalline nature of CuNPs as shown in **Fig. 3b**.

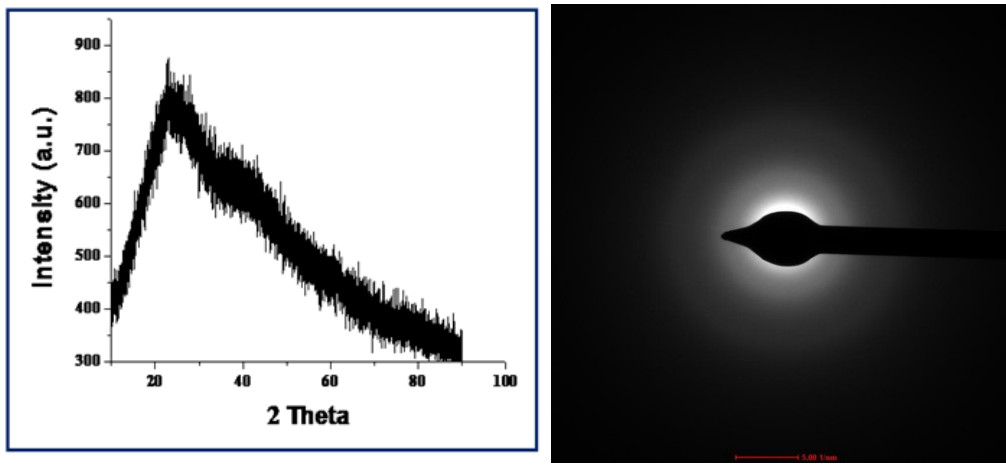

Fig.3: (a) XRD spectra of biosynthesized CuNPs (b) SAED pattern of biosynthesized CuNPs

Biosynthesized CuNPs were further scanned using SEM (Fig. 4) and showed less aggregated particles with nearby spherical shape. CuNPs were assembled on the surface due to the interactions such as hydrogen bonding, Vander Waal's force of attraction and electrostatic interactions between the bio-organic capping molecules bound to the CuNPs.

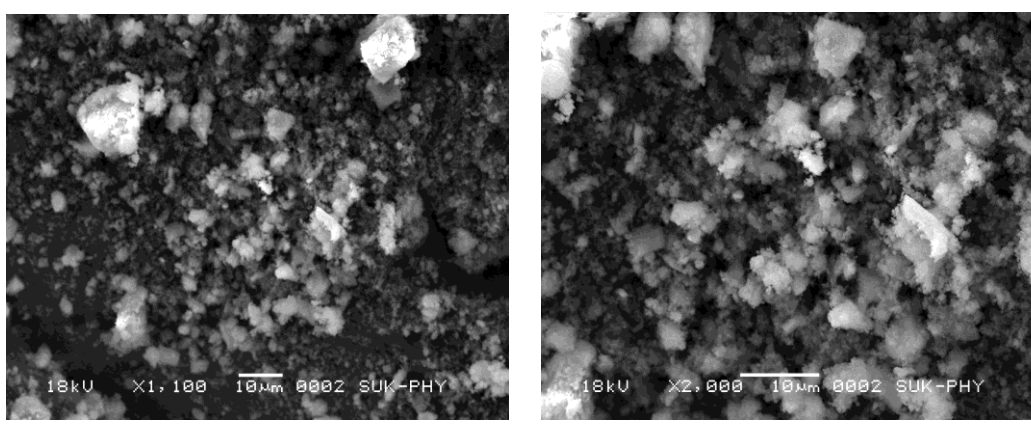

Fig.4: SEM images of biosynthesized CuNPs

The uniformity and particle size of biosynthesized CuNPs were investigated using TEM analysis. The particles are distributed in the range 65-75 nm. According to the electron micrographs, the CuNPs is predominantly oval in shape with an average particle size of 69 ± 3 nm. The presence some larger particles can be attributed to the aggregation or overlapping of smaller particles.

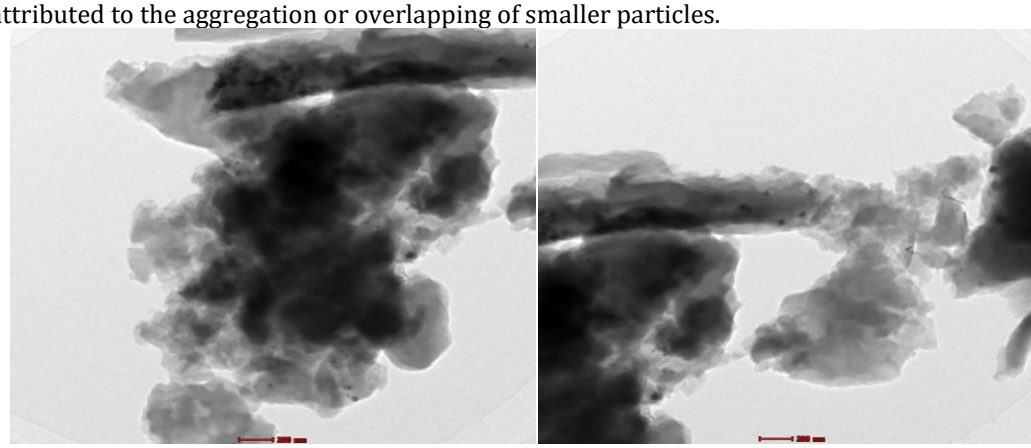

Fig.5: TEM images of biosynthesized CuNPs

IV. Conclusion

We have reported green, ecofriendly, simple one pot methodology for the synthesis of CuNPs by using aqueous leaves extract of *Tribulus terrestris* as a reducing as well as stabilizing agent at ambient temperature without adding external reducing agent or surfactant template. XRD and SAED analysis showed that the biosynthesized CuNPs nanomaterial was amorphous in nature where as the TEM analysis revealed that the size of CuNPs was ~ 69 nm.

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Environment benign degradation of Chloramphenicol- A Review

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ABSTRACT: *Antibiotics and their metabolites are released constantly in aquatic environments. Conventional treatment methods are not able to degrade them completely because they are persistent against biological degradation and natural attenuation. It has resulted in their frequent presence in the wastewater. Advanced oxidation processes found more effective than conventional methods for degradation of chloramphenicol.*

Keywords: *antibiotics, metabolites, degradation*

I. Introduction

The presence of antibiotics in environment has attracted the researchers due to high consumption, low biodegradability, toxic effects and contribution to the development of resistant bacteria in aqueous systems. Their presence in environment increases tremendously due to several sources such as release during production, generation of domestic and hospital waste, human and animal excretion. The kinetic of chloramphenicol oxidation has been studied both spectrophotometrically and iodometrically by several investors. In this article would like to consolidate the various work done on the well known antibiotic drug that finds extensive application in pharmaceutical industries in the last few decades.

The oxidation kinetics of this drug by oxidants like organic haloamine, metal ion oxidants, use of catalyst, variation in media, become useful to understand the mechanism of metabolic conversion of chloramphenicol in biological system. It is also helpful to identify the reactive species of the oxidant in aqueous acid/base. The degradation is also reported by advanced oxidation processes like degradation by solar radiation, UV/ persulfate system, UV/ H_2O_2, thermally activated persulfate. The results of various studies are interpreted and consolidated.

Chloramphenicol (commercial name Chloromycetin) is a synthetic antibiotic derived firstly from the bacterium Streptomyces venezuelae, isolated by David Gottlieb. Chloramphenicol (CAP) was introduced into clinical practice in 1949. CAP shows strong effectiveness against gram-positive and gram-negative bacteria including mostly anaerobic microorganisms as well as rickettsias and certain large viruses. It is a bacteriostatic antimicrobial mainly used for the treatment of a variety of infections of the skin, ear and ophthalmic (eye-drops) including trachoma and veterinary purposes [1-3]. Recent studies indicate that, CAP alone or in combination with other anticancer drugs can cause inhibition of growth in several cancer lines including leukemic cell line.CAP was used in treating acute fever, dysentery, typhoid, meningitis, plague and pneumonia fever [4].

The presence and accumulation of chloramphenicol antibiotics in aquatic environments may cause threats to ecosystem and human health by inducing increase and spread of bacteria drug-resistant due to long term exposure. This leads to development of the various advanced oxidation processes for the transformation of CAP in water.

This review includes following research work:-

1] Oxidation by Hexacyanoferrate(III) in alkaline medium

The review on the above titled study gave a knowledge on the various steps to be followed in the kinetic study, catalytic effect on rate constant and mechanism of the reaction. A comparative kinetics study of uncatalysed and Ru(III)-catalyzed oxidation of chloramphenicol by hexacyanoferrate(III) in alkaline medium has been investigated spectrophotometrically.

Ionic strength maintained was 1.10 mol dm^{-3}. The reaction exhibit 1:2 stoichiometry in both cases [5]. Oxidation takes place via electron transfer process resulting in the formation of free radical intermidiate. The order with respect to [CAP] was less than unity. The rate constants increased with increase in [alkali] and order was found to be less than unity in catalyzed (0.64) and uncatalyzed (0.57) reaction.

About the oxidant: Hexacyanoferrate(III) ion is powerful one electron oxidant with redox potential of +0.45V in alkaline medium.

About the catalyst:- In recent years the use of transition metal ions such as ruthenium, osmium, palladium

either or alone or as binary mixtures are preferred in redox reactions[6].

Kinetic study:-UV-Visible spectrophotometric study was done under pseudo-first order conditions where [CAP] > [HCF(III)] in both the uncatalyzed and catalyzed reactions. The progress of the reaction was followed at λ_{max} 420 nm.

Product analysis :- The main oxidation product formed with 1 mole of CAP and 2 mole of HCF(III) at constant ionic strength after 5h in closed vessel was identified as p-nitrobenzaldehyde (confirmed by IR and H^1 NMR spectra).

2] Oxidation by Chloramine –T in acidic medium

The review reports oxidizing property of Chloramine–T on CAP in acidic medium iodometrically. The use of catalyst was emphasized in reported study. The oxidation was very slow in aqueous solution and became appreciable in presence of H^+ ions at higher temperature.

About the oxidant :- Chloramine-T is the member of haloamine family. It is good oxidizing agent in both acidic and alkaline media. CAT, a byproduct in saccharin manufacture is well known as analytical reagent, and the mechanistic aspects of its reaction have been well documented[7-9]. CAT undergoes two-electron change in its reactions resulting in the formation of the product p-tolune sulphonamide and sodium chloride. Depending on the reaction conditions, it can behave as both electrophile or nucleophile.

About the catalyst:- Ruthenium (III) chloride is well known non-toxic and homogenous catalyst [10,11]. The study explored the catalytic role of Ru (III) with N-halo compounds as oxidant.

Kinetic study:- The progress of the reaction was followed iodometrically at 313K.The oxidation of CAP in acidic medium was studied under pseudo-first order condition. Determination of unconsumed CAT in the reaction mixture shows 1:2 (CAP:CAT) stoichiometry[12].Reaction is zero order with respect to CAP and first order with respect to CAT and Ru(III). Solvent polarity and dielectric constant has no significant effect on reaction. The fractional order dependence of rate on $[H^+]$ suggests complex formation between oxidant and $[H^+]$.The proposed mechanism is supported by the derived reaction order.

3] Oxidation by 1-chlorobenzotriazole in acidic medium

Kinetic study was performed under pseudo first order conditions with excess of the CAP over the oxidant at 323K[13].

About oxidant :- CBT is flexible oxidizing agent and its solution chemistry is surely known [14]. It can be utilized as oxidizing as well as chlorinating agent. It is a novel potential oxidant because of its reactivity towards various functional groups [15]. The reduction product of CBT is benzotriazole (BTA) and HCl.

Product-The main oxidation product is aldehyde (confirmed by IR). The reduction product is benztriazole.

4] Oxidation by Ceric ammonium sulphate (CAS) in mixed solvent media

The change in microbial activity of CAP with respect to oxidation of alcoholic groups has been followed by CAS in acetic acid-water 50% (v/v) media[16].

Kinetic study:- The oxidation was found to be first order with respect to [CAS] and fractional order with respect to [CAP].

Oxidant :- Cerium salts are considered to be conventional oxidant used to oxidize carbon compound in various acid media via the formation of 1:1 cerium (IV)-substrate complex which undergo dissociation unimolecularly in the rate determining step.

Product analysis: The product of reaction is ketonic acid(confirmed by FTIR and NMR). The stoichiometry of CAP-Cerrium(IV) was found to be 1:4.

5] Degradation by thermally activated persulfate in aqueous solution

This study aimed to evaluate the technical feasibility of degradation of CAP by thermally activated persulfate(TAP).

Kinetics : CAP degradation followed a pseudo-first order kinetics. CAP degradation rate constants (k_{obs}) increased with increased temperature and sodium persulfate dosage [17]. A lower pH resultated in a greater increase in CAP degradation and the highest degradation efficiency was obtained at pH 2.96. CAP removal efficiencies of 62.2-96.3% in the wastewater matrices by TAP were achieved within 160 min.

Product analysis:- The CAP concentration in aqueous samples were measured using a high-performance liquid chromatography. The intermediate products formed were analyzed by GC/MS after 0,10,20,40,60,80,120 and 160 min. treatment. Eleven intermidiates produced during the TAP oxidation process were identified and a primary reaction mechanism was proposed.

Notably, TAP oxidation could be considered as an efficient method of decreasing the load of CAP in polluted water.

6] Degradation of chloramphenicol by sodium alginate as a polymer template in presence of ZnO nanosphere.

The photodegradation of CAP is achieved through the generation of electron hole pair. The photodegradation was found to increase up to 50 mg of catalyst and thereafter there was no significant increase[18].

About oxidant: ZnO nanospheres have been prepared by hydrothermal method in presence of sodium aliginate as a template. The fluorescence spectrum of the nanospheres shows a band gap excitation emission, the intensity of which decreases in presence of drug molecules. Such decrease indicates the interaction between the drug molecules and the species obtained as a result of excitation of an electron from the conduction band to the valance band of ZnO.

About catalyst: Zno nanospheres are found to be an efficient heterogeneous photocatalyst in presence of UV light of wavelength 365nm.The method of synthesis is simple.

Product analysis:- Initially two intermidiates were predicted due to loss of either dichloroacetic acid moiety or denitration. Removal of dichloroacetic acid moiety leads to 4-(2-Amino-1,3-dihydroxy-propanyl)-nitrobenzene. Denitration generates 2,2-dichloro-N-[1,3-dihydroxy-1-(4-hydroxyphenyl)-propan-2-yl]-acetamide. Both intermidiates react with photo generated hydroxyl radicals and holes leading to complete mineralization. The degradation was confirmed by measuring the total organic content(TOC).

Chloramphenicol was found to degrade within 180min. The mechanism proposed involves the formation of electron hole pair leading to the formation of reactive species.

7] Oxidation by metaperiodate using Rh(III) catalyst

The kinetics of oxidation of CAP by $NaIO_4$ in presence of Rh(III) catalyst has been investigated spectrophotometrically in alkaline medium at 308K.

The objectives of work are to ascertain the influence of the oxidant, substrate and the medium on the reaction kinetics, explore the catalytic activity of Rh(III) on the oxidation of CAP by sodium metaperiodate, identify the oxidation products during the course of the reaction and also elucidate a plausible mechanism and appropriate rate law.

Kinetic study: The reaction shows first order kinetics with respect to [IO_4^-] and [Rh(III)] and fractional order kinetics with respect to [alkali] and [CAP]. Ionic strength and dielectric constant of the medium show no effects on the reaction rates.

Product analysis: Reaction products are p-nitrobenzaldehyde, 2-amino-2-hydroxyethanol, dichloroacetic acid. Stoichiometry of reaction after 72h was found to be 1 mol of CAP for 2 mol of $NaIO_4$ [19].

About oxidant:- It is powerful oxidizing agent, widely employed as a diol cleaving agent[20]. Sodium metaperiodate is less potent oxidant in alkaline media than in acidic media.

About catalyst: It is widely used as catalyst due to strong catalytic influence in various reaction. It is non toxic and homogeneous catalyst.

8] TiO_2 assisted photocatalytic degradation of chloramphenicol

The photo-oxidation of CAP followed pseudo-first order kinetics in presence of TiO_2.

About catalyst: It is most studied catalyst due to its biological and chemical resistance, low price safe to use, affordability. But its recycling is expensive.

Photo-catalytic oxidation was reported to give best results during first 40-60 min. treatment. The further irradiation did not result in the total removal of CAP from water.

Product analysis:- During the first 60min the removal of CAP proceeded almost linearly and then there observed a small decrease of [CAP]. The products of photo-oxidation are glycolic aldehyde, dichloroacetamide and p-nitrobenzaldehyde which are resistant to further oxidation [21].

The time dependence of treatment of CAP revealed that the best change in the water was observed during first hour of treatment. The photo-oxidation of CAP proceeds faster over smaller crystals of TiO_2 and the photo-catalysts with enhanced surface area were more effective than their low surface area counterparts. As photo-oxidation products are resistant to further oxidation, the photo-catalytic treatment using TiO_2 can't totally solve the environmental hazards.

9] Photolytic degradation of chloramphenicol in different aqueous matrices using artificial and solar radiation

The photo-degradation of CAP in ultrapure water(UW), untreated surface water(USW), and treated effluent from sewage treatment plant(TESTP) in laboratory scale and piolet scale, was evaluated using solar and artificial radiation[22].

Kinetic study: CAP degradation followed pseudo-first order kinetics with apparent degradation rate constants(k_{app})following the order UW=USW> TESTP. The k_{app} and half-life were strongly influenced by the radiation source. The higher k_{app} and lower $t_{1/2}$ of CAP using artificial radiation in comparison with the solar

experiments is due to the high irradiance furnished by the higher pressure to the solar experiments.

Product analysis : The CAP transformation products were analysed by liquid chromatography/ triple quadrupole mass spectroscopy(LC-MS/MS), in negative ionization mode. Using 15 and 20V ions with m/z 78,121,152,176 and 257 were obtained. Reducing fragmentation intensity was obtained for 10 and 5V, the ions with m/z 121 and 78 disappeared and ions formed from deprotonation of CAP was appeared (m/z 321).Mono and di-hydroxyl transformation products were identified in UW after 40 min of solar irradiation.

The application of a combined treatment is necessary to guarantee the total mineralization of CAP and transformation products, preventing deleterious effects in aquatic system.

II. Conclusion :

Antibiotics and their metabolites are released constantly in aquatic environments. Conventional treatment methods are not able to degrade them completely because they are persistent against biological degradation and natural attenuation. It has resulted in their frequent presence in the wastewater.

Both spectrophotometric and iodometric methods were reported in the past decade for oxidation of chloramphenicol. Media maintained plays an important role in the oxidation behavior of CAP. Some studies require high temperature while some require use of catalyst in the reaction. The review is also helpful to identify the reactive species of the oxidant in aqueous acid/base. Advanced oxidation processes found more effective than conventional methods.

Into the environment, elimination of antibiotic can occur by biotic (biodegradation) or non-biotic (sorption, hydrolysis, photolysis, oxidation and reduction) processes. Antibiotic are difficult to remove by biotic processes. Non-biotic elimination depends on the physicochemical properties of the target compound. Among these alternatives photolysis can be considered the main process of elimination of pollutants in surface water. Photo-degradation efficiency depends on conditions such as temperature, pH, composition of matrix and radiation source.

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Synthesis and characterization of ZnOnanorod by CBD method

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ABSTRACT: *A simple, easy to handle, inexpensive chemical route of chemical bath deposition method is used to synthesize ZnOnanorod at room temperature. In synthesis of ZnOnanorod thin films, 0.1M solutions of ZnCl2 was used as source of zinc ion. Triethanolamine was used as complexing agent in precursor solution and supersaturated this solution by diluted NaOH solution. Formation of ZnOnanorod is confirmed by XRD pattern of annealed films. SEM micrographs of ZnO depicts nonrod like morphology. EDAX is employed to confirm elemental composed of ZnO and 3.06eV band gap was recorded by the photoluminescence spectroscopy.*

Keywords: *Chemical bath deposition method, XRD, SEM, EDAX and Photoluminescence etc.*

I. INTRODUCTION

Recently, solid-state semiconductor based on metal oxides have been widely used for various purposes. Many other oxides like CdO, In_2O_3, WO_3, ZnO, SnO_2 andCeO_2, have been examined my many researchers [1-7] Besides this, stability of material, cheapness, controlled industrial or any professional use are the big challenges in this field. With such challenges, it is possible to improve the electrical and optical properties of materials by changing the surface properties, such as electronic band gap, O vacancies, crystal deficiencies and specific surface area (SSA). Therefore, such systems are becoming more and more important in material field and are being used as photovoltaics, electrochromics, sensors, photocatalysts and anti-ultraviolet agent [8-10]. Among many metal oxides, zinc oxide (ZnO) has attracted considerable attention because of its low cost, non-toxicity, high stability and high efficiency.

Specifically, zinc oxide nanoparticles can serve as an excellent source for resisting bacterium and shielding ultraviolet [11-12]. Currently, however, the highly efficient use of ZnO is impaired by its wide bandgap (3.27eV), which only responses to a small fraction of the sun's energy spectrum. Thus, one of the goals to improve the optical response of ZnO is to increase its optical activity by doping with special atom. Recently various methods of preparation are attracting the attention to overcome such problems. Surface modification of semiconductors can be achieved by addition of second component and used as active sites for redox processes and as promoting free charge carriers that increases the electronic conductance of oxide film [13].

In the present work, we have synthesized ZnOnanorod by chemical Bath Deposition (CBD) method. Structural morphological, compositional and optical properties of ZnO are investigated by means of XRD, SEM, EDAX and photoluminiscence analysis.

II. EXPERIMENTAL

In this work, ZnOnanorod was synthesized by simple and inexpensive CBD method. Initially, Glass substrates were washed with same procedure as mentioned in our previous work [14]. Afterward 1.32 gm of Zinc Chloride ($ZnCl_2$) was dissolved in 100 ml double distilled water to prepare 0.1M solution of Zinc chloride. Then Triethanolamine solution was added drop wise in it. Sodium hydroxide (NaOH) solution was added drop wise into this freshly prepared solution with continuous stirring and made the solution supersaturated till the clear transparent solution was obtained. Further it was named as ZZ. Finally this solution bath was kept at room temperature along with vertically inserted previously washed glass substrate without any disturbance. White coloured deposition was obtained on glass substrates after 48 hours. This film was air dried for 2-3 hrs. Then substrates were subjected for annealing at temperature at 500˚C for 2 hour and Annealed sample was further applied for characterization. To study the structural properties, surface morphological studies, compositional properties of annealed films were investigated by X-ray diffraction patterns using X-ray diffractometer over, scanning electron microscopy (SEM), Energy Dispersive X-ray Analysis (EDAX) and Photoluminescence spectroscopy.

III. RESULT AND DISCUSSION

3.1 Formation mechanism

The synthesis of ZnO by Chemical Bath Deposition (CBD) is based on the formation of solid phase from the solution, which includes two steps as nucleation, and particle growth. These two steps are represented in the form of reaction mechanism as in eq (1-4)

Step I

$ZnCl_2 + H_2O \longleftrightarrow [Zn^{2+} + 2(Cl^-)] + [2H^+ + O^{--}]$ .....(1)

$[Zn^{2+} + 2(Cl^-)] + TEA \longleftrightarrow [Zn(TEA)]^{2+} + 2Cl$ .....(2)

$[Zn(TEA)]^{2+} + 2NaOH \longleftrightarrow Zn(OH)_2 + 2Na + TEA$ .....(3)

Step II

$Zn(OH)_2 \xleftrightarrow{\text{Annealing}} ZnO + H_2O$ .....(4)

3.2 X-Ray Difraction

The structural changes and identification of phases of ZnO thin films obtained by CBD are investigated with the help of X-ray diffraction (XRD). Synthesized sample was characterized by Philips automated X-Ray diffractometer (PW-3710) equipped with crystal monochromator employing Cu-Kα radiation of wavelength 1.5406 A°. The diffracting angle (2θ) is varied between 20°- 80° and the recorded XRD patterns for the thin film is shown in **Fig 2.** peaks of 31.81°, 34.54°, 36.29°, 47.53°, 56.58°, 62.85° and 67.92° are corresponding to (100), (002), (101), (102), (110), (103) and (112) respectively. Sample ZZ shows hexagonal wurtzite crystal structure. No reflections due to any secondary phase are detected in the XRD pattern. The obtained XRD spectra matched with the [JCPDS file No.36-1451]. This confirms hexagonal wurtzite crystal structure of ZnO.

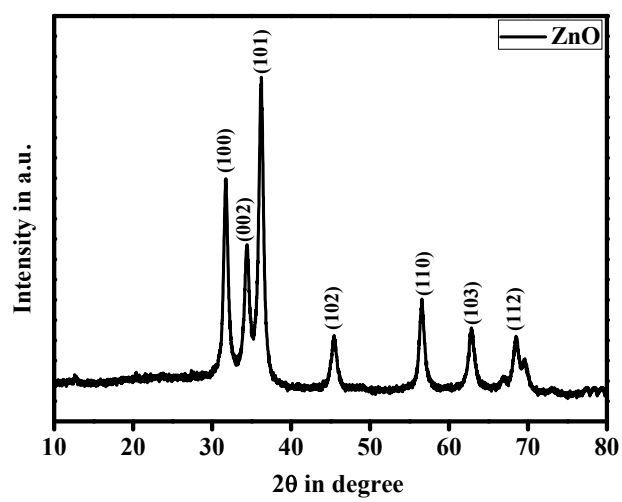

Fig 1: XRD of ZnONanorod

3.3 SEM and EDAX

SEM micrographs of ZnOfilm is shown in **Fig 3** The morphology of the annealed sample was measured by field-emission scanning electron microscopy (FESEM, JSM-7001F, JEOL). Scanning electron microscope image of (a)–(d) sample ZZ shows interconnected hexagonal nanorods morphology respectively. In **Fig 3 (c)**, SEM image shows magnified SEM image along with their dimensions of sample ZZ. These SEM images are observed at resolution of x 10,000, at constant voltage 15.0 kV. The minimum and maximum length of ZnOnanorod is 1.40 and 2.52µm in length respectively and its average length is 1.78 µm. Whereas ZnOnanorod is hexagonal in shape as shown in **fig 3 (d)** and it has average hexagonal side length is 238 nm. However such types of morphologies are useful in various applications. Elemental composition is confirmed by energy dispersive X-ray analysis. From **Fig. 4**and **Table 1**it is obvious that elemental composition is in good agreement with the stoichiometric ratio of elements we had maintained during experiment.

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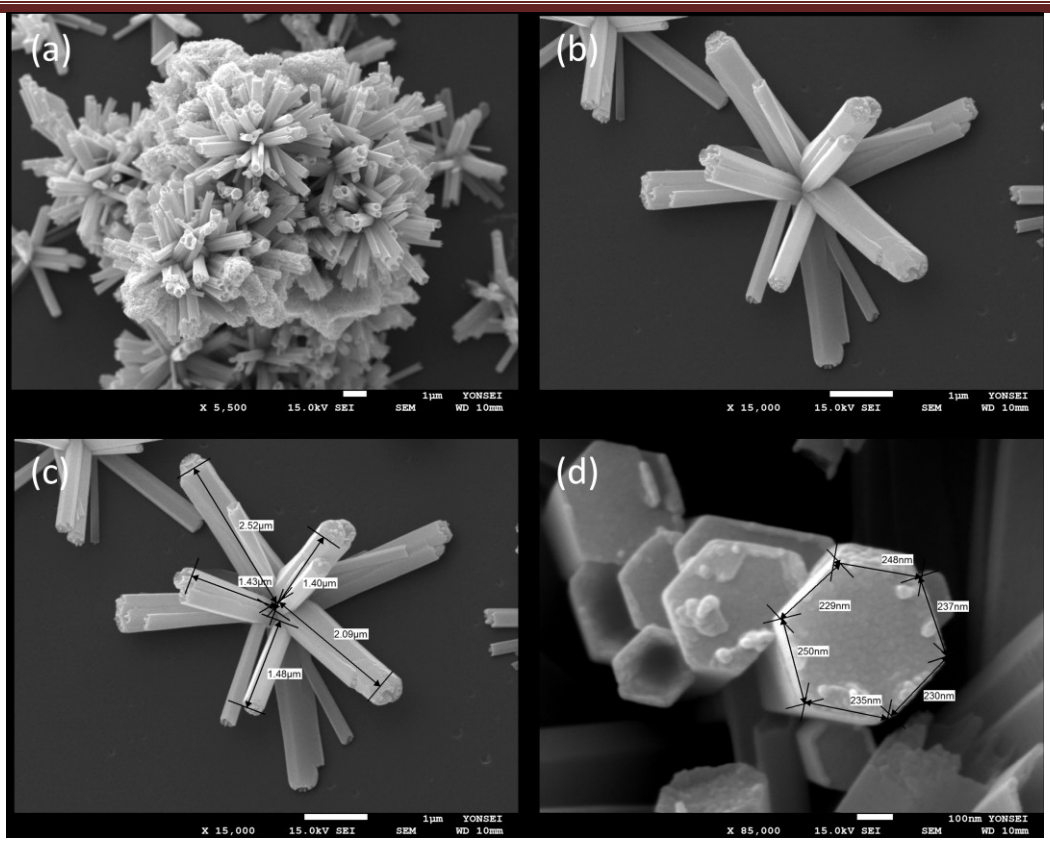
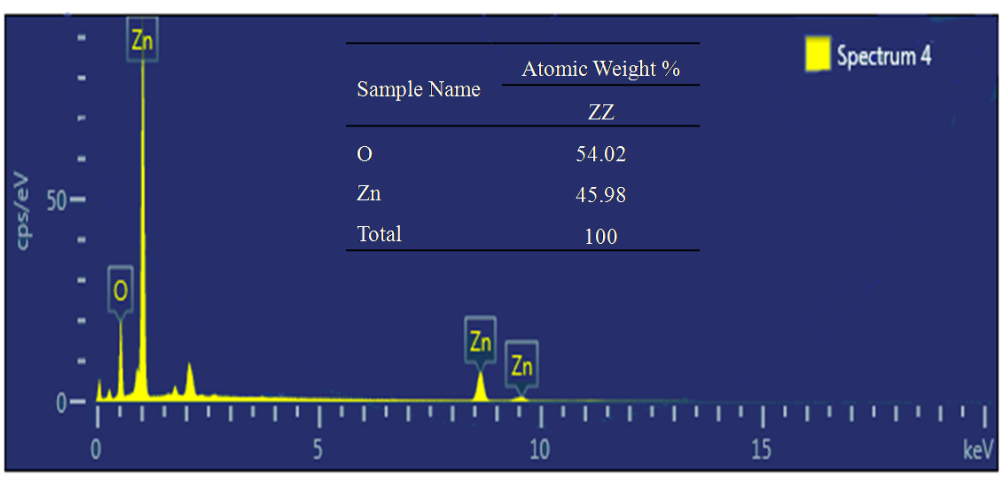


Fig 2: SEM micrograph of ZnOnanorod.



Sample Name	Atomic Weight %
	ZZ
O	54.02
Zn	45.98
Total	100

Fig 3: EDAX of ZnOnanorod and inset showing a table of elemental composition of ZnO

3.4 Photoluminescence

Fig. 4 shows PL spectra for sample ZZ was investigated at room temperature. The results indicates that the response of the PL spectra have two emitting bands including a weak emission band in UV region. In general visible spectrum observed is associated with structural defects. Maximum intensity was observed at 404 nm for ZZ hence its bandgap is recorded as 3.06eV. A strong emission band in visible region for sample ZZ is observed. The UV emission band originates from the direct recombination of the free excitons through an exciton–exciton collision process, while the emission peaks are due to radial recombination of the photo-generated hole with the electrons that belongs to the singly ionized oxygen vacancies [15-16]

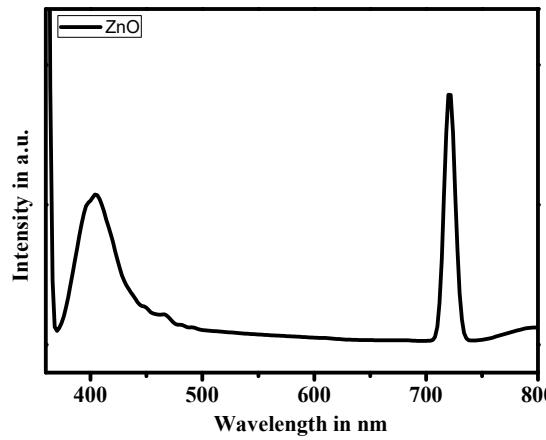


Fig 4: Photoluminescence of ZnOnanorod.

IV. CONCLUSION

Synthesized chemical bath deposition method was employed successfully for synthesis of ZnOnanorod. XRD patterns confirmed the polycrystalline crystal structure of ZnO, SEM analysis of the samples showed nanorod-like morphology. Elemental compositions were confirmed by EDAX analysis. Photoluminescence study showed maximum intensity at wavelength 404 nm for ZZ. The observed band gap is recorded as 3.06eV.

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CASHMERE BOUQUET LEAVESA SIMPLE AND AFFORDABLE SOURCE AS A NATURAL INDICATOR FOR ACID-BASE TITRATION

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ABSTRACT: *The Indictor is an essential part in laboratory procedures. The acid base indicators so far used in schools and colleges are Phenolphthalein, Methyl red and Methyl orange. The present work represents the non conventional plant materials to be used as acid base indicator. The aqueous extract of Cashmere Bouquet leaves were tested for pH and used as indicator for Strong acid- Strong base, Strong acid -Weak base and Weak acid-Strong base titrations. The performance was found comparable to that of conventional acid base indicators. The accuracy of end points was tested using volumetric methods. It was found very useful, economical, simple and accurate indicator for said titrations.*

Keywords: *Cashmere Bouquet, Acid base titrations, indicators*

I. Introduction

The Nature shows its Creativity on this planet in various life forms. Each creation is unique and distinguished from the other. The design of leaves amazing color combinations shapes and sizes leave us in wonder. It Provide calmness to the eyes and their sweet fragrance spread positivity in the air. They contribute in making food items, scents and even medicines. Leaves also attract the insects for pollination. Thus leaves are miracle of the nature, for the world. *Cashmere Bouquet* is a species of flowering plant in the family Verbenaceae[1]. *Cashmere Bouquet* is a beautiful perennial shrub its origin is china, Japan. It grows to heights of 5' to 8', has large downy mid-to-dark green leaves, and leaves in tight clusters that are very fragrant in the evening, Leaves season is spring through summer zones 9b to 11. This plant does not need a lot of fertilizer in fact over-feeding will cause it to grow excessively leafy at the expense of blooms. The plant has neither thorns nor tendrils the best time for pruning is November[2]. In present study, we used *Cashmere Bouquet* leaves extract as natural and effective indicator for acid-base titration[3-5].

II. MATERIAL AND METHODS

Fresh leaves of *Cashmere Bouquet* were collected from the local gardens of Kolhapur regions, Maharashtra, and they were authenticated from R. C. Shahu college botany department, Kolhapur. All other ingredients were of analytical grade and purchased from Loba Chemie Pvt Ltd, Mumbai. Reagents and volumetric solutions were prepared as per standard books [6,7]. The leaves were cleaned by distilled water and cut into small pieces and macerated for 20 min. in 25ml of 90% ethanol. The extract was preserved in tight closed container and stored away from direct sun light.[7] The experiment was carried by using the same set of glassware's for all types of titrations. As the same aliquots were used for both titrations i.e. titrations by using standard indicators and leaves extract, the reagents were not calibrated. The equimolar titrations were performed using 25 ml of Titrand with three drops of indicator. All the parameters used for Analysis and the Comparison of Color Change are given in **Table 1**. A set of three experiments each for all the types of acid base titrations were carried out. The mean and standard deviation for each type of acid base titrations were calculated from results obtained.

TITRATIONS

The developed indicator tested for all three types of acid base titration viz. strong acid vs. strong base (HCl Vs NaOH), weak acid vs. strong base (CH_3COOH Vs NaOH) and strong acid vs. weak base (HCl Vs. NH_4OH). The sharp end point was observed for all types.

III. RESULT AND DISCUSSION

The leaves extract was screened for its use as an acid base indicator in various acid base titrations, and the results of this screening were compared with the results obtained by standard indicators methyl red and phenolphthalein. The results of these titrations are given in **Table 2**.

The leaves extract of *Cashmere Bouquet* was found to have Anthocyanin and is pH sensitive. The

results of pHchanges in various acid base titrations of this leaves indicator are shown in **Table 1.** The pH value checked for the leaves extract of *Cashmere Bouquet* and it was found as pH 7.25 Also the color and pH change observed during acid base titrations is more significant over standard indicator as it gives a sharp color change at equivalence point thus the result obtained showed that the routinely used indicators could be replaced successfully by leaves extract as they are simple, accurate, economical and precise.

Table-1: Parameters Used For Analysis and the Comparison of Color Change

Titrant	**Titrand**	**Indicator Color Change** (Phenolphthalein) (pH range)	**Indicator Color Change** (Methyl red) (pH range)	**Indicator Color Change** (Leaves Extract) (pH range)
HCl	**NaOH**	Pink to Colorless (13.12 -7.85)	Pale yellow to Brown (13.13 -7.21)	Yellow to Colorless (13.05 to 7.76)
HCl	**NH_4OH**	Pink to Colorless (10.42 -7.91)	Yellow to brown (10.43 -7.22)	Yellow to colorless (10.36 -7.06)
CH_3COOH	**NaOH**	Pink to Colorless (13.09 -7.75)	Yellow to brown (13.10 -7.24)	Yellow to colorless (13.60 -7.31)

HCl: - Hydrochloric Acid, **NaOH**: - Sodium Hydroxide, **NH_4OH**: - Ammonium Hydroxide, **CH_3COOH**: - Acetic Acid,
Leaves Extract- *Cashmere Bouquet*.

Table-2: Results of Screening.

Sr. No	**Titration (Titrant v/s Titrand)**	**Strength (Moles)**	**Indicator**	**Mean (ml) S.D. (±)**	**Color change (pH range)**
1	**NaOH V/S HCl**	0.1	Methyl red Leaves extract	25.1 ± 1.000 25.0 ± 1.830	Yellow to Red(13.25 - 6.57) Yellow to colorless(13.52 - 6.81)
		0.5	Methyl red Leaves extract	25.2 ± 1.596 25.1± 1.296	Yellow to Red (12.90 - 5.55) Yellow to colorless (13.15 - 5.89)
		1.0	Methyl red Leaves extract	24.0 ± 1.173 24.8 ± 1.141	Pale yellow to brown (13.) Yellow to colorless (13.14 - 2.23)
		0.1	Phenolphthalein Leaves extract	25.0 ± 1.292 25.1 ± 1.294	Pink to colorless (13.76 -7.34) Yellow to colorless (13.52 - 7.81)
		0.5	Phenolphthalein Leaves extract	24.9 ± 1.829 25.1 ± 1.296	Pink to colorless (12.53 -7.23) Yellow to colorless (13.15 - 5.89)
		1.0	Phenolphthalein Leaves extract	27.6 ± 1.173 27.9 ± 1.122	Pink to colorless (13.72 - 7.74) Yellow to colorless (13.05 - 2.23)
2	**HCl V/S NH_4OH**	0.1	Methyl red Leaves extract	25.1 ± 1.830 25.0 ± 1.827	Yellow to Red (10.90 - 6.55) Yellow to Colorless (10.63 -7.04)
		0.5	Methyl red Leaves extract	24.9 ± 1.838 25.1 ± 1.833	Yellow to Red (10.96 - 6.29) Yellow to Colorless (11.09 - 6.59)
		1.0	Methyl red Leaves extract	24.8 ± 0.122 24.8 ± 0.122	Yellow to Brown (10.58 - 6.35) Yellow to Colorless (10.68 - 5.75)
		0.1	Phenolphthalein Leaves extract	25.1 ± 1.296 25.2 ± 1.827	Pink to colorless (10.15 - 7.80) Yellow to colorless (10.70 -7.50)
		0.5	Phenolphthalein Leaves extract	25.0 ± 1.292 24.8 ± 1.300	Pink to colorless (11.51 - 8.11) Yellow to colorless (11.07 - 6.57)
		1.0	Phenolphthalein Leaves extract	24.3 ± 0.115 24.9 ± 0.239	Pink to colorless (11.43 - 7.90) Yellow to colorless (10.68 - 5.75)
3	**CH_3COOH V/S NaOH**	0.1	Methyl red Leaves extract	24.9 ± 1.141 24.7 ± 1.794	Yellow to Red(11.64 - 6.04) Yellow to colorless (10.78 - 6.15)
		0.5	Methyl red Leaves extract	24.7 ± 1.271 24.3 ± 1.294	Yellow to Red (12.55 - 6.30) Yellow to colorless (12.57 - 6.58)
		1.0	Methyl red Leaves extract	25.8 ± 1.141 25.3 ± 1.141	Yellow to Brown (13.77 - 7.23) Yellow to colorless (13.79 - 6.80)
		0.1	Phenolphthalein Leaves extract	24.7 ± 1.816 24.9 ± 1.284	Pink to colorless (10.85 - 6.45) Yellow to colorless (10.78 - 6.45)

		0.5	Phenolphthalein Leaves extract	24.2 ± 1.786 24.4 ± 1.260	Pink to colorless (12.55 - 8.07) Yellow to colorless (12.57 - 6.52)
		1.0	Phenolphthalein Leaves extract	25.5 ± 1.212 25.3 ± 1.141	Pink to colorless (13.83 - 8.07) Yellow to colorless (13.79 - 6.80)

S.D. (±) - standard deviation

IV. CONCLUSION

For all types of titrations equivalent point obtained by the Leaves extract either exactly coincide or very close with the equivalent point obtained by standard indicators. Thus natural indicator employed in the acid base titrations was found economic, safe and an efficient alternative for traditional indicators. In comparison to this, chemical indicators were found more expensive and hazardous, which proves that Leaves extract of *Cashmere Bouquet* as a natural indicator is more worthy.

V. ACKNOWLEDGEMENTS

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Extraction and separation of bismuth(III) using liquid anion exchanger from aqueous hydrochloric acid media

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ABSTRACT: *Bismuth(III) is quantitatively extracted with 0.6 M N-n decylaniline in xylene from 1.5 M hydrochloric acid media. Bismuth(III) laden organic phase was stripped by acetate buffer and bismuth(III) determined by spectrophotometrically. N-n hexylaniline, N-n –heptylaniline, N-n octylaniline have also been studied as an extractant for bismuth(III) but result found are more satisfactory for N-n decylaniline. Xylene, toluene, benzene, cyclohexane, carbon tetrachloride have been studied as diluents for amine out of which xylene is most efficient. The proposed method used for diverse ion study; the separation of bismuth(III) from binary mixtures as well as ternary mixture are also carried out.*

Keywords: *Bismuth, Solvent Extraction, N-n decylaniline.*

I. Introduction

Bismuth metal is relatively inert soft and malleable. The world production of bismuth is about 5000 Tons per year [1]. Bismuth is seen the least toxic, heavy metal for human being and chiefly employed in medical application for its good antimicrobial properties, it have demulcent properties that why it used in gastrointestinal disorders [2] it forms low melting alloy which are massively employed in fire suppressor equipment [3]. Bismuth and its alloys are frequently added to molten iron steel and aluminum alloys. The utility of bismuth telluride in thermoelectric cooling and low temperature thermoelectric power production. Bismuth is work as carrier as well as coolant in liquid metal fuel reactor [LMFR] [4]. Bismuth and its compound performs key role in synthesize of cosmetic product and semiconductor [5] due to voluminous application extraction and separation of bismuth(III) is of analytical importance. Liquid –liquid extraction is prominent technique used for recover of bismuth(III) by using liquid anion exchanger N-n octylaniline [6-8] and Aliquat 336 [9] in mineral acid media. Tri-n-octylamine [10] N-n-hexylaniline has been used as extractant in sulphuric acid media [11] extraction is done by using N,N,N´N´-tetraoctyl-3-oxapentanediamide (TODGA) from nitric acid to n-dodecane [12] The important organophosphrous compound is used as extractant such as Bis(2,4,4-Trimethylpentyl) phosphinodithioic acid (Cyanex 301) [13], Bis(2,4,4-trimethylpentyl) monotiophosphinic acid (Cyanex 302) [14]. Bismuth(III) is extracted with highly acidic solution of H_2SO_4 using Cyanex 925 [15] tributyl phosphate TBP used in hydrochloric acid medium [16] Spectrophotometric and atomic adsorption spectrometric method are employed for determination of fluoroquinoloneantibacterials by ion pair complex formation with bismuth(III) tetraiodide. [17]. Bismuth iodide complex is extracted by using (MIBK) and determination on (AAS) [18] formerly we analysis quantitative extraction of thorium by using N-N heptylaniline and platinum group metal with 2-octylaminopyridine [19-22]

II. Experimental

All the chemicals are utilized analytical grade and purchased from Aldrich. pH of the solution was measured on Elico digital pH meter LI-120. Standard bismuth(III) solution was prepared by dissolving adequate amount of bismuth nitrate in concentrate HNO_3 and diluted to 1 liter double distilled water and standardized [23]. By using gardlund method, N-n –decylaniline was synthesizes and its solution was prepared by in xylene.

2.1 General extraction and procedure

Solution containing 1 mg of bismuth(III), add sufficient quantity of HCl to make the concentration of 1.5 M in a total volume of 15 ml. Transferred the solution into a 125 mL separating funnel and shaken the solution for 3 minutes with 10 mL of 0.15 M N-n-decylaniline. Mixture was mixed vigorously and both the layer was allowed to separate. The organic phase was stripped twice with 25 mL of acetate buffer. Estimation of bismuth(III) in stripped solution was carried out via complexometrically.

2.2Influence of amine concentration on extraction of bismuth(III)

Impact of amines concentration on extraction behavior of bismuth(III) was investigated. In

order to improve the extraction condition of bismuth(III) N-n-decylaniline in xylene with change in concentration from 0.038 M to 0.22 M were used; according to results it was observed that 10 ml of 0.15 M N-n-decylaniline was plenty for 1 mg of bismuth(III) extraction from 1.5 M HCl (Fig. 1).

2.3 Influence of acid concentration on extraction of bismuth(III)

Impact of acid concentration on extraction of Bi(III) was studied. The extraction is carried out from various mineral acids like hydrochloric acid, hydrobromic acid, hydroiodic acid, sulphuric acid, perchloric acid, nitric acid, acetic acid (Fig. 2). At various concentration of acid the extraction of Bi(III) increases with increasing acid concentration. The quantitative range was the 0.7 M to 2.0 M further increase in acid concentration the extraction of Bi(III) decrease. While other acid shows incomplete extraction. Hence, 1.5 M hydrochloric acid concentration was used throughout experiment to ensure the complete extraction.

2.4 Influence of various diluents on extraction of bismuth(III)

At constant other parameters the Bi(III) was extracted with 0.15 M N-n-decylaniline with various diluents benzene, MIBK, kerosene, toluene, n-butanol, amyl alcohol, amyl acetate, carbon tetrachloride to name a few (Table 1). The quantitative extraction was observed in xylene, toluene, benzene, amyl acetate, carbon tetrachloride, MIBK while other diluents shows results as n-butanol (55.55 %) and chloroform (22.22 %). Therefore, the cost and availability is concern xylene was preferred for further study.

Table: 1 Influence of diluents on Bi(III) extraction

Diluent	Dielectric constant	Percentage extraction (%E)	Distribution ratio (D)
Xylene	2.30	99.9	999
Benzene	2.28	99.9	999
Toluene	2.38	99.9	999
Carbon-tetrachloride	2.24	99.9	999
Chloroform	4.81	22.22	4.12
Nitrobenzene	34.8	85	5.66
Amy acetate	5.0	99.9	999
Methyl isobutyl Ketone	13.10	99.9	999
n-butanol	17.51	55.55	4.55
Diethyl ether	4.33	99.9	999

Condition: Bismuth(III) = 1 mg, N-n decylaniline = 0.15 M in xylene, HCl = 1.5 M aqueous: organic = 1:1, shaking time = 3 min, strippant = acetate buffer (2×20 mL).

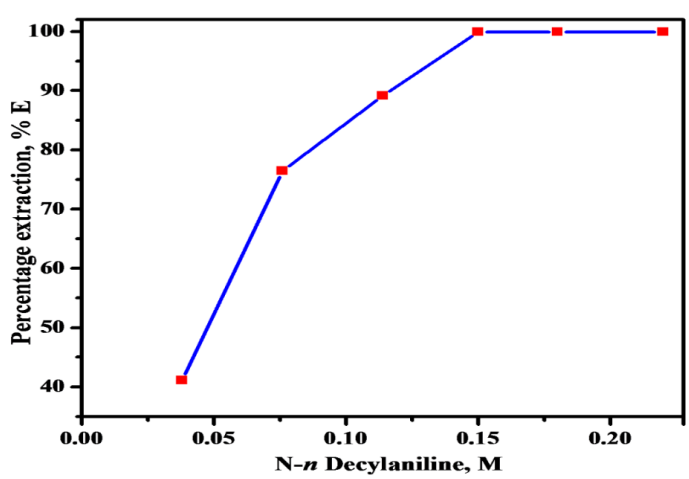

Figure 1

2.5 Influence of aqueous to organic volume ration

The effect of aqueous to organic volume ratio has been studied for the optimization of quantitative extraction. The ration was varied from 1:1 to 20:1 out of which the complete extraction was found up to ratio 2.5: 1. Hence for next step of extraction the ration 2.5:1 was preferred (Table 2).

Table: 2 Influence of aqueous to organic volume ratio on extraction of Bi(III)

Aqueous: organic	Percentage extraction, (%E)	Distribution ratio, (D)
1:1	99.9	999
2:1	99.9	999
2.5:1	99.9	999
5:1	75	3
7.5:1	75	3
10:1	62.5	1.66
15:1	62.5	1.66
20:1	37.5	0.6

Condition: Bismuth(III) = 1 mg, N-n decylaniline = 0.15 M in xylene, HCl = 1.5 M, aqueous: organic = 1:1, shaking time = 3 min, strippant = acetate buffer (2×20 mL)

2.6 Influence of equilibration time

Variation of shaking time from 0.25 min to 15 min was investigated, the result shows that the extraction was quantitative throughout the study from 1 min. Initially the extraction was not quantitative but as time increase from 0.25 to 1 min the extraction was quantitative which remains constant upto 15 min. Hence for further study 2 min equilibrium time was adopted.

2.7 Influence of loading capacity of N-n-decylaniline

The loading capacity of 10 ml of 1.5 M N-n decylaniline was determined by varying the concentration of Bi(III) from 0.5 mg to 7.0 mg. N-n-decylaniline quantitatively extract Bi(III) upto 3 mg, further increase in the quantity of Bi(III) results in the decrease in extraction. So the recommended procedure continued with 1 mg of Bi(III).

2.8 Influence of stripping agent

The back stripping of the metal from loading organic phase is significant in commercial extraction task hence various stripping agent tested for the stripping of Bi(III) from loading N-n-decylaniline. The stripping of Bi(III) was found to be quantitative with acetate buffer (2×25 ml) solution while other stripping agent such as Nitric acid, sulphuric acid, perchloric acid, acetic acid, back stripping was incomplete (Table 3).

2.9 Influence of temperature

The influence of temperature is crucial study in extraction process. Bismuth(III) extraction from aqueous solution was accomplish at 0.15 M N-n-decylaniline with 1.5 M HCl media in xylene at changing temperature between 298 to 328 K. It was observed that temperature increase, distribution coefficient increase. Vant hoof equation indicate the change of the extraction equilibrium constant (k_{ex}) D (log K_{ex})/d(1/T)= ($\Delta H/2.303R$) The plot of log Kex*Vs*1000/T is liner with slope -2.08 (Fig. 3) the enthalpy change of extraction process is endothermic entropy ΔS and free energy ΔG were calculated by equation

$\Delta S = \Delta H - \Delta G/T$

$\Delta G = - 2.303\ RT \log K_{ex}$

Negative value of ΔG propose reaction is spontaneous and positive enthalpy value confirm extraction of Bi(III) increase with increasing temperature Table 4.

Table: 3 Influence of strippant on extraction of Bi(III)

Strippant (M)	H_2SO_4		HNO_3		$HClO_4$		CH_3COOH	
	%E[a]	D[b]	%E[a]	D[b]	%E[a]	D[b]	%E[a]	D[b]
0.1	32.0	0.47	23.0	0.29	48.0	0.92	11.0	0.12
0.3	34.0	0.51	28.0	0.38	54.0	1.17	24.0	0.31
0.5	38.0	0.61	47.0	0.88	70.0	2.33	36.0	0.56
0.7	0	-	59.0	1.43	78.0	3.54	40.0	0.66
0.9	0	-	77.0	3.34	80.0	4	46.0	0.85
1.0	0	-	89.0	8.09	80.0	4	54.0	1.17
2.0	0	-	85.0	5.66	74.0	2.84	58.0	1.38
3.0	0	-	81.0	4.26	66.0	1.94	58.0	1.38
4.0	0	-	77.0	3.34	64.0	1.77	52.0	1.08
5.0	0	-	71.0	2.44	56.0	1.27	48.0	0.92

Condition: Bismuth(III) = 1 mg, N-n decylaniline = 0.15 M in xylene, HCl = 1.5 M aqueous :organic =1:1, shaking time = 2 min, % E[a] = Percentage extraction, D[b] = Distribution ratio

Table: 4 Influence of temperature on extraction of Bi(III)

Temperature (K)	1000/T	Percentage extraction (%E)	D	Log D	Log K_{ex}	ΔG ($KJmol^{-1}$)
298	3.356	54.0	1.173	0.070	0.246	-1403.63
303	3.300	63.5	1.739	0.240	0.418	-2425.06
308	3.246	65.0	1.857	0.269	0.447	-2636.10
313	3.194	69.5	2.278	0.358	0.535	-3206.28
318	3.144	72.5	2.636	0.420	0.599	-3647.18
323	3.095	82.0	4.555	0.659	0.837	-5176.44
328	3.048	84.5	5.451	0.737	0.915	-5746.44

Table: 5 Influence of diversion on extraction of Bi(III)

Foreign ion	Added as	Tolerance limit, (mg)
Ba (II)	$BaCl_2.2H_2O$	30.0
Ni(II)	$NiCl_2.6H_2O$	30.0
Cr(VI)	$K_2Cr_2O_7$	30.0
Zn(II)	$ZnSO_4.7H_2O$	25.0
Sr(II)	$Sr(NO_3)_2$	20.0
Ga(III)	$GaCl_3$	5.0
Th(IV)	$Th(NO_3)_4.5H_2O$	10.0
Pb(II)	$Pb(NO_3)_2$	5.0
Hg(II)	$HgCl_2$	5.0
Mg(II)	$MgCl_2.6H_2O$	5.0
Co(II)	$CoCl_2.6H_2O$	5.0
Mn(VII)	Mn_2O_7	5.0
Sn(II)	SnO	5.0
Te(IV)	TeO_2	5.0
Sb(III)	Sb_2O_3	5.0
Mo(VI)	MoO_3	5.0
Nd(III)	Nd_2O_3	5.0
Fe(III)	$FeCl_3$	5.0
Ag(I)	$AgNO_3$	5.0
Al(III)	$AlCl_3.6H_2O$	5.0
In(III)	$InCl_3.4H_2O$	5.0
Ascorbate	L-Ascorbic acid	100
Malonate	Sodium malonate (trihydrate)	100
Acetate	Sodium acetate	100
Succinate	Sodium succinate	100
Thiourea	Thiourea	100
Tartrate	Sodium tartrate	100
Bromide	Potassium bromide	20.0
Iodide	Potassium iodide	100
Nitrate	Ammonium nitrate	100
Nitrite	Sodium nitrate	100
Phosphate	Disodium hydrogen phosphate	100
Fluoride	Sodium fluoride	15.0

Table: 6 Separation of Bi(III) from binary mixture

Metal ion	Concentration, mg	Average recovery, %
Bi(III)	1	99.5
Zn(II)	1	97.3
Bi(III)	1	98.5
Pb(II)	1	96.3
Bi(III)	1	99.2
Cd(II)	1	98.7
Bi(III)	1	99.6
Mg(II)	1	98.3
Bi(III)	1	99.6
Ni(II)	1	98.9
Bi(III)	1	99.8
Al(III)	1	99.1
Bi(III)	1	99.4
Sb(III)	1	97.8
Bi(III)	1	99.3
Cu(II)	1	98.5
Bi(III)	1	99.1
Ba(II)	1	98.6
Bi(III)	1	98.9
Cr(VI)[b]	1	98.1
Bi(III)	1	98.1
Sn(IV)[c]	1	96.6
Bi(III)	1	98.5
Tl(I)	1	97.8

[b]Useof 1 ml 0.5 M NH_2OH, HCl for reduction of Cr(VI) to Cr(III) before extraction
[c]Masked by 5 mg F^-

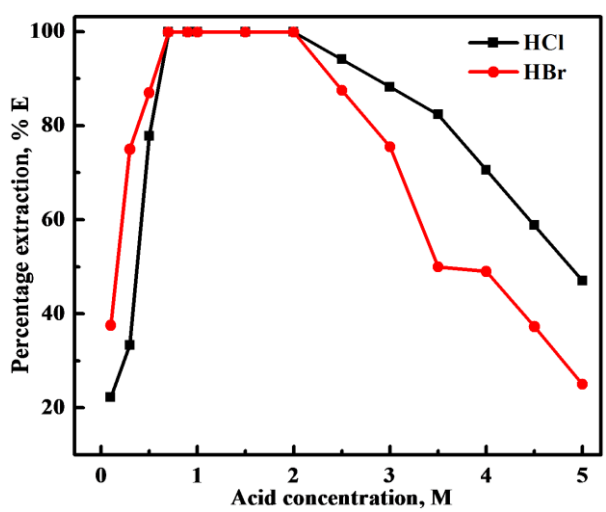

Figure 3

III. Applications

3.1 Influence of diverse ion

The influence of different anions and cations on the extraction of Bi(III) with 0.15 M N-n-decylaniline at 1.5 M HCl was studied within relative error 2%. The result illustrate other metal ions was nil and interference of cation can be reduced by using proper masking agent (Table 5)

3.2 Separation of Bi(III) from binary mixture

The utility of the proposed method was suitable for isolation and determination of bismuth(III) from number of binary mixture. Bi(III) was successfully separated from its associated metal ion very

few metal ion interfered which are masked by proper masking agent each associating ion was determined by specific method while Bi(III) was determined complexometrically by EDTA which is shown in table 6.

3.3 Separation of Bi(III) from ternary mixture

Separation of Bi(III) from ternary mixture containing generally associated and toxic metal. Associated metal ion remains in aqueous phase where asBi(III) from organic phase was stripped with acetate buffer and determined complexometrically table 7.

3.4 Analysis of Bi(III) from pharmaceutical samples

This method is permitted to determination of Bi(III) in pharmaceutical samples. such as Trymo tablet A tablet was dissolved in concentrated perchloric acid and solution was evaporated to near dryness repeatedly the residue was then filtrate with distilled water and finally diluted to 100 ml with distilled water calculate amount of solution was used for analysis of Bi(III) by recommended procedure six time replicate analysis was done the amount of Bi(III) find out by our procedure table 8.

3.5 Analysis of synthetic mixture corresponding to the composition of ore samples

The effectiveness of this method was prove by extraction of Bi(III) from synthetic mixture corresponding to ore sample. Result of triplicate analysis indicates that Bi(III) could be separated and determined from the ore sample table 9.

IV. Conclusion

N-*n*-heptylaniline showed a good potential for the extraction of bismuth(III) from HCl media. The developed method is very simple and selective. Bismuth (III) is free from interference of large number of foreign ions which are commonly associated with bismuth (III) in its natural occurrence. It is applicable to the analysis of bismuth(III) in synthetic mixtures and pharmaceutical samples.

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A lemon juice catalysed synthesis of quinoxaline derivatives: as a green approach

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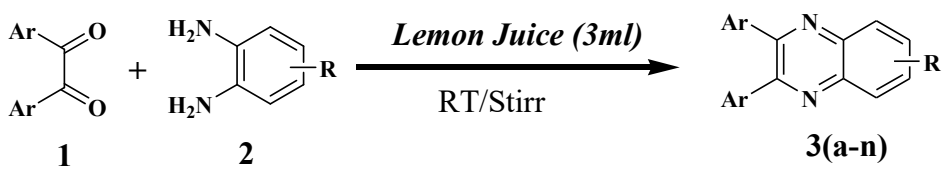
ABSTRACT: *In the present investigation, we have developed an efficient and greener protocol for the synthesis of quinoaxalines via two component one-pot condensation between benzil and orthophenylenediamine (OPD) under Lemon juice as a catalyst. Lemon juice catalyst was found to be highly efficient, inexpensive, environmentally benign, non -toxic and ecofriendly. This solvent free approach was completely nonpolluting having several advantages such as mild reaction condition with good to excellent yield in short reaction time with simple workup procedure.*

Keywords: *Natural Catalyst, Solvent free approach, Non-chromatographic Technique, Quinoxalines.*

I. Introduction

Family of quinoxaline skeleton exhibit the source of some bactericides,[1] antitumor agents,[2] herbicides,[3] insecticides,[4] fungicides.[5] Also, they are used in dyes,[6] building blocks for the synthesis of organic semiconductors,[7] cavitands,[8] DNA cleaving agents,[9]dehydroannulenes, [10] and electrical-photochemical materials.[11] Literature data reveals that various catalytic systems were employed for the synthesis of substituted quinoxalines. Most common method relies on the condensation of 1,2-diamines with α-diketones under microwave irradiation,[12] and the use ofzeolites,[13] $H_6P_2W_{18}O_{62}.24H2O$, and ionic liquids[14] as a catalyst. Conversely, most of the traditional processes have no agreement with the green chemistry protocols which limit their use under the aspect of environmentally benign processes[15].

Considering these facts and in continuation of our interest in application of naturally sourced material for organic transformation[16]. Herein we wish to report environmentally benign protocol for the synthesis quinoxalines using *Citrus limon*juice as green catalyst **(Scheme 1)**. *Citrus aurantium, Citrus indica, Citrus limonium*are some important species of citrus family commonly known as lemon. The juice is highly soluble in water and thus acts as a homogeneous catalysis on solvent free synthesis of quinoxaline.



Scheme 1

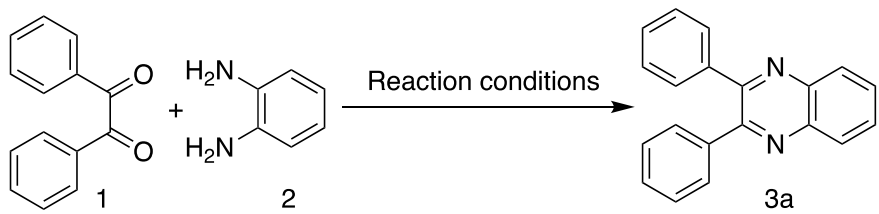
The main ingredients of the extract of *Citrus limonium*species of lemon are minerals (0.3%), ascorbic acid or vitamin-C (0.5%), fat (0.9%), protein (1%), fibres (1.6%), citric acid (5-7%), carbohydrates (11.2%), moisture (85%) and some other organic acids[16d]. The juice is soluble in water. Due to presence of ascorbic acid and citric acid, lemon juice is acidic (pH= 2-3) in nature, and thus it works as acid catalyst in organic reactions.Conventional uses of lemon juice are cooking, industrial and medicinal. Nowadays the lemon juice plays important role of catalyst in organic synthesis.

II. RESULTS AND DISSUSION

Fresh lemon was collected from home garden in Tasgaon area, washed with water cut by using knife and then pieces were pressed in a fruit juice to get the juice extract. Then the juice was filtered through filter paper to remove solid material and to get clear juice which is used as a catalyst.

Initial studies were undertaken using the two component one-pot reaction of benzil and orthophenylenediamine based as a model reaction **(Scheme 2)**. We examined the model reaction with 3 ml

pineapple juice as a catalyst under neat condition at room temperature. The reaction proceeded to offered the desired functionalized quinaxoline in modarate yield (61.79%) (Table 1, entry 2). In order to enhance the yield, the reaction condition is optimized using model reaction under different catalyst such as Lemon, Orange, Grapes, checkpea and papaya. The observed results are summarized in table 1. As shown in Table 1, it was found that the yields are normally observed under the influence of fruit juice from moderate to good. This method works good with grapes juice, pineapple juice and lemon juice in shorter reaction time compaired with papaya juice and cheakpea juice. From table 1 it was clear that lemon juice is best catalyst for synthesis of desired derivative.



Scheme 2. Model reaction

Table 1. Screenings of natural catalyst.

Sr. No.	Catalyst[a] (3 ml)	Time (hr)	Yield[b] (%)	pH	
				Before	After
1	-	05.00	NR	-	-
1	Pineapple	03.10	61.79	3.30 - 3.60	6.80
2	Lemon	02.50	82.76	2.00 - 2.60	3.10
3	Orange	11.30	74.74	3.69 - 4.34	5.90
4	Grapes	07.00	70.14	2.90 - 3.82	4.51
5	Checkpea	05.15	52.83	2.30-2.40	3.10
6	Papaya	05.00	55.14	5.77	8.45

NR: No Reaction
[a]Heating at RT with constant stirring of benzil (1mmol), orthophenylenediamine (1mmol), and lemon juice for appropriate time.
[b]Isolated yield.

The effect of catalyst amount on the multicomponent reaction was investigated by varying the catalyst amount (1, 2, 3, 4 5, 10 ml). It was found that when increasing the amount of the juice from 1, 2, 3, 4, 10 ml, the yields also increased (Table 2). A further increasing of the catalyst loading (above 3 ml) does not affect the yield. Therefore, 3 ml of grapes juice is sufficient to push this reaction forward. Next, we investigated the reusability of lemon juice. At the end of the reaction, the catalyst could be recovered by a simple filtration. After the second run, the yields were gradually decreased (70 and 60% respectively, for third and fourth runs).

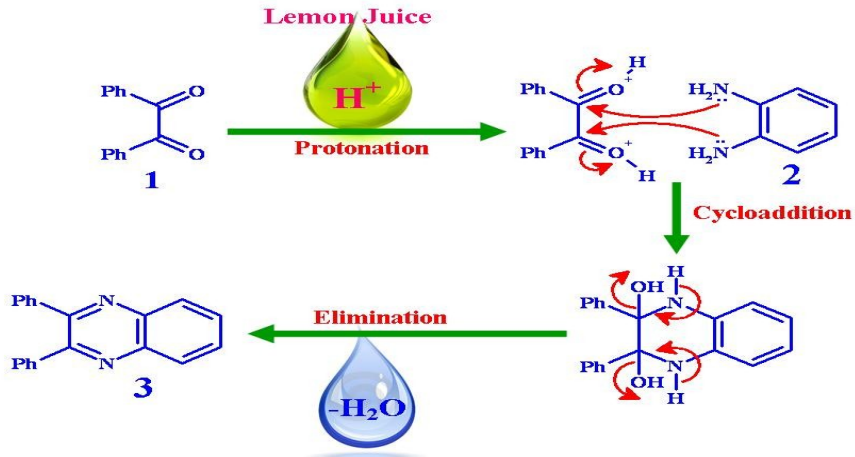
Table 2. Optimization of lemon juice catalyst

Entry	**Reaction Condition[a]**	**Time (hr)**	**Yield [b] (%)**
1	Lemon juice (1 ml)/ RT/Stirr	3.5	86
2	Lemon juice (2 ml)/ RT/Stirr	2.5	92
3	Lemon juice (3 ml)/ RT/Stirr	2.0	96
4	Lemon juice (4 ml)/ RT/Stirr	2.0	96
5	Lemon juice (5 ml)/ RT/Stirr	2.0	90
6	Lemon juice (10 ml)/ RT/Stirr	2.5	88
7	Citric acid (2 mol %)/water (2ml)/RT/Stirr	4.5	90

[a]Heating at RT with constant stirring of benzil (1mmol), orthophenylenediamine (1mmol), and lemon juice for appropriate time.
[b]Isolated yield.

Although we did not investigate the reaction mechanism, the plausible mechanism for the quinoxaline formation is depicted in **Scheme 3**. The carbonyl diketone is activated by hydrophilic acidic groups present in lemon juice. The hydrophobic part of juice may be increases the driving force of diamine

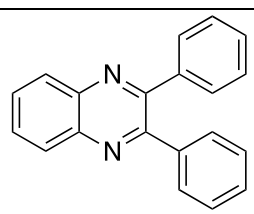
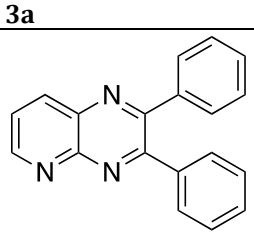
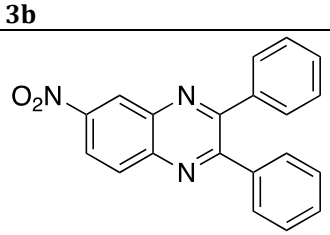
to interact with activated diketone and eliminated water molecules get easily absorbed by the hydrophilic part of catalyyst. As a result, of the overall effect there is a rate enhancement of the reaction.

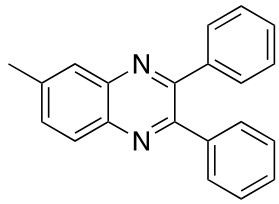
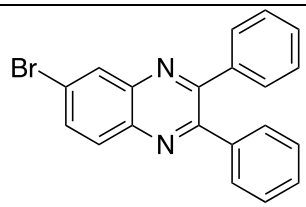
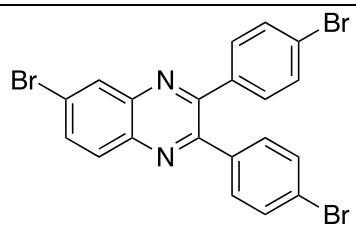
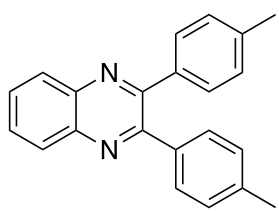
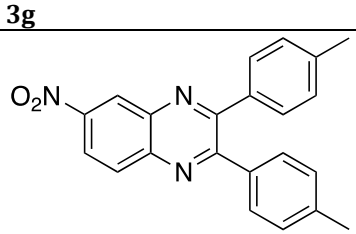
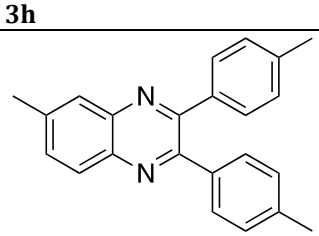
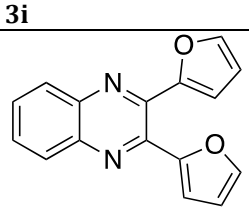


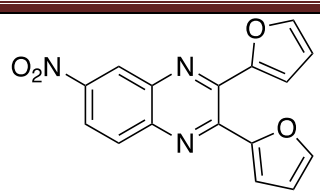
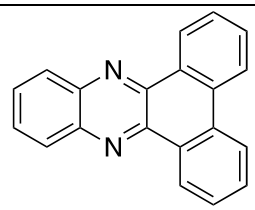
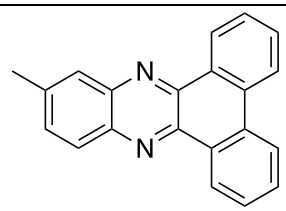
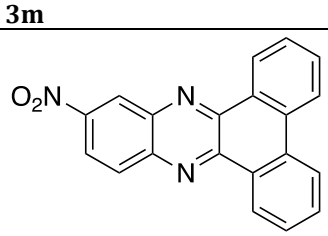
Scheme 3. Postulated role of lemon juice catalyst in the formation of quinoxalines

Encouraged by the remarkable results obtained with the above motioned reaction conditions and to show generality and scope of this new protocol, we extended these optimized protocol for the preparation of range of quinoxaline and the results are given in **Table 3**. In all cases, *o*-phenylenediamine with different substituents carrying either electron-donating or electron-withdrawing groups reacted successfully and gave the expected products in high yields and short reaction times.

Table 3. Lemon Juice catalyzed synthesis of functionalized quinaxolinederivatives[a]

Sr. No.	Product (3a-n)	Time (hr)	Yield (%)	Melting point (°C)	
				Obs.	Rep.[ref.]
1	 **3a**	2.0	96	127-130	126-127[15]
2	 **3b**	1.5	90	142-144	142-143[15]
3	 **3c**	2.0	92	184-186	185-186[15]

4	 **3d**	1.5	93	134-136	133-135[15]
5	 **3e**	1.0	90	155-158	158-160[15]
6	 **3f**	2.0	94	230-235	236-237[15]
7	 **3g**	3.0	90	146-148	146-147[15]
8	 **3h**	2.0	91	192–194	191-193[15]
9	 **3i**	1.5	92	220-222	225-227[15]
10	 **3j**	2.5	88	130-132	131-132[15]

11	 **3k**	2.0	91	172-174	171-173[15]
12	 **3l**	2.5	86	223-225	224-226[15]
13	 **3m**	3.0	87	218-220	217-219[15]
14	 **3n**	2.5	82	238-242	240-242[15]

[a]Reaction condition: 1, 2-diketones (1.0 mmol), aromatic 1, 2-diamines (1.0 mmol), lemon juice (3 ml) stir at room temperature.
[b]Isolated yield.

III. EXPERIMENTAL SECTION

All reactants were obtained from Merck and Aldrich company and used without further purification. IR spectra were obtained using potassium bromide pallets on Bruker ALPHA FT-IR Spectrometer. All NMR spectra were recorded on 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR spectrophotometer using tetramethylsilane (TMS) as an internal standard and $CDCl_3$ as solvent. The Mass Spectra were measured on a Shimadzu GC/MS-Q5050A spectrometer. Melting points were determined on open capillary method on DBK-programmable melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was run on Merck silica gel 60 F_{254} plates to determination of purity of the substrates as well as to monitor the reactions.

3.1 Typical procedure for the synthesis of 2,3-diphenylquinoxaline(3a)

A 25 mL round bottomed flask was charged with the benzil (1 mmol), *o*-phenylenediamine (1 mmol), lemon juice catalyst (ml). The whole reaction mixture was stirred at room temperature for the stipulated period of time **(Table 3)** till the completion of the reaction (monitored by TLC). After completion of reaction, the reaction mixture was cooled to room temperature and diluted with water (10 mL) and again stirred for 5 min, then the reaction mixture was filtered to recover the catalyst and the filtrate was concentrated under vacuum. The crude product was purified by recrystallization with 96% ethanol to give the pure product **3a**. Authenticity of the synthesized products **(3a-n)** has been confirmed by FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopic techniques.

3.2 *Spectral characterization of some synthesized derivatives*

2,3-Diphenylquinoxaline (Table 1, entry 1): ^{13}C NMR (75 MHz, $CDCl_3$): δ 127.29, 128.89, 129.12, 129.91, 130.06, 138.92, 141.11, 153.38 ppm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33(bs, 6H, Ar-H) 7.54(bs, 4H, Ar-H)

7.74(bs, 2H, Ar-H) 8.20(bs, 2H, Ar-H) ppm ; FT-IR (KBr): 1662 cm^{-1} (stretching C=N); MS: *m/z* = 282 (M+).

2,3-Diphenylpyrido[2,3-b]pyrazine(Table 1, entry 2):
^{13}C NMR (75 MHz, $CDCl_3$): δ 125.64, 128.58, 128.83, 129.72, 129.86, 130.23, 130.67, 136.60, 138.51, 138.94, 150.27, 154.49, 155.15, 156.75 ppm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.347.45 (m, 5H), 7.59 (d, 2H), 7.66 (d, 2H), 7.737.76 (dd, 2H), 8.548.56 (dd, 1H), 9.20 (d, 1H) ppm; FT-IR (KBr): 1638 cm^{-1} (stretching C=N); MS: *m/z*= 283 (M+).

6-Nitro-2,3-diphenylquinoxaline (Table 1, entry 3): ^{13}C NMR (75 MHz, $CDCl_3$): δ 123.269, 125.512, 128.450, 129.667, 129.854, 129.953, 130.666, 137..950, 139.870, 143.390, 147.801, 155.621, 156.176 ppm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38(bs, 6H, Ar-H) 7.56(bs, 4H, Ar-H) 8.28(bs, 1H, Ar-H) 8.45(bs, 1H, Ar-H) 9.02(bs, 1H, Ar-H) ppm; FT-IR (KBr): 1656 cm^{-1} (stretching C=N); MS: *m/z* = 327 (M+).

6-Methyl-2,3-diphenylquinoxaline (Table 1, entry 4): ^{13}C NMR (75 MHz, $CDCl_3$): δ21.948, 128.040, 128.244, 128.663, 128.731, 129.903, 129.915, 132.321, 139.246, 139.728, 140.486, 141.289, 152.552, 153.289 ppm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.61(s, 3H, Ar-CH3) 7.35(s, 6H, Ar-H) 7.55(d, J=6.48, 4H, Ar-H)7.60(s, 1H, Ar-H) 7.98(s, 1H, Ar-H) 8.09(d, J=8.4, 1H, Ar-H) ppm; FT-IR (KBr): 1619 cm^{-1} (stretching C=N); MS: *m/z* = 296 (M+).

IV. CONCLUSION

We developed straightforward cost-effective, green and efficient approach for the synthesis of quinoxaline derivatives by the *in situ* oxidation and condensation of aliphatic and aromatic 1,2-diketones with various 1, 2-diamines using natural lemon juice as a promoter was developed with high yields at room temperature. To the best of our knowledge this is the first report of the synthesis of quinoxalines using lemon juice and this new reaction conditions open an important alternative to the use of toxic solvents.

V. ACKNOWLEDGEMENTS

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KINETIC STUDY OF OXIDATIVE DEGRADATION OF P-CHLORO-SUBSTITUTED AROMATIC CARBOXYLIC ACID HYDRAZIDE BY VANADIUM (V)

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ABSTRACT: *Chemical kinetic study of oxidative degradation of p-chloro- substituted aromatic carboxylic acid hydrazide by Vanadium (v) was studied in aqueous ethanol medium under pseudo first order condition. The formation of complex between the reactants, which decomposes in the subsequent step to give corresponding aromatic carboxylic acid. Transfer of single electron with intervention of free radical was observed during the course of the reaction. The effect of different initial [V(v)] on reaction rate shows constancy in k values which indicates the, pseudo-first order kinetic behavior of the reaction. Increase in hydrazide concentration decreases the specific rate. The rate of reaction is directly proportional to the Concentration of the acid as well as dielectric constant of the medium of the reaction. There is no effect of change in Concentration of salt as well as various salts of the same concentration under the experimental conditions on the reaction rate.. The variation of ionic strength during the course of the reaction has negligible effect on rates of reactions. The study of effect of temperature on the rate of the reaction was done in between 30 to 55 ^{o}C and the specific reaction rates are found to be directly proportional to the increase in temperature.. The activation parameters were determined and the values support the proposed mechanism as evidenced by considerable decrease in entropy of activation.($-\Delta S^{\#}$ = 144.98, J K^{-1} mol^{-1}), The progressive study of the reaction was followed by measuring the absorbance (A) of the V(v) at 410nm.*

Keywords: *p-chloro- substituted aromatic carboxylic acid hydrazide, Ammonium metavanadate, Oxidative degradation, pseudo-first order, Thermodynamic parameters.*

I. Introduction

Hydrazides are obtained by preparing derivatives of Carboxylic acids and hydrazines[1]. It's multiple use in various branches of chemistry[3,4,5,6,7,8]especially pharmaceutical Chemistry[2], needs to study the mechanism of their oxidation in detail. The process of oxidation reaction is an important transformation in an organic chemistry. The formation of corresponding acids[9,10,11,12,13,14,15,16] is observed during the most of the oxidation reactions of hydrazine's. Literature survey shows that, the chemical kinetic study of oxidative degradation of substituted aromatic carboxylic acid hydrazides by vanadium (v) is not extensively studied. Therefore kinetic study of p-chloro- substituted aromatic carboxylic acid hydrazide by vanadium (v) was undertaken. The study of Chemical kinetics deals with the rate at which the chemical reactions occur and the influence of various factors such as concentration, temperature, pressure catalysts etc.on the reaction rates. Different chemical reactions occur at different rates.

II. MATERIAL AND METHOD

The anhydrous NaHCO3, H_2SO_4, salt, ammonium metavanadate (AR grade), hydrazine hydrate (BDH 99%) and fresh distilled ethanol was used. Ethyl ester of p-Chloro substituted aromatic carboxylic acid was prepared by esterification[17] andconverted to correspondinghydrazide by using the prescribed procedure[18].An equimolar mixture of ethyl ester of p-Chloro substituted aromatic carboxylic acid and hydrazine hydrate (B.D.H. 99%) was refluxed for 15 minutes. Then enough absolute ethanol was added through the condenser to get clear solution and further refluxed for more than six hours. The excess of hydrazine hydrate, solvent ethanol and other unreacted material was removed by distilling the solution under reduced pressure. The resulting hydraziderecrystallised from ethanol and stored in amber colored bottles and kept in dark place. The distilled water obtained by redistillation of distilled water in the presence of a few crystals of $KMnO_4$ and a few pellets of KOH using Borosil glass distillation assembly was used throughout the experiment. The 0.01 M solution of oxidant ammonium metavanadate was prepared by dissolving accurately calculated and weighed quantity of ammonium metavanadate in hot double distilled water using Pyrex glass measuring flask. The standardization of V(v) solution was done by titrating it against standard $Fe(NH_4)_2SO_4$ solution by using diphenylamine as an indicator. Similarly the stock solution

of $NaClO_4$ was prepared by dissolving equivalent quantities of Na_2CO_3 and $HClO_4$(70% E.Merck) in H_2O to maintain ionic strength. Standard p-chloro-benzoic acid hydrazide solution was prepared by dissolving it in aqueous alcoholic medium.

2.1 Determination of λ max for the reaction:

The absorption maxima ie λ max of the oxidation reaction under study was determined by varying wavelength, it was observed that maximum absorbance for both ammonium metavanadate and a mixture of hydrazide and ammonium metavanadate was obtained at 410 nm λmax.

2.2 Method of following the kinetic reaction:

The oxidant and substrate are taken in separate conical flasks along with required quantities of sulphuric acid and sodium perchlorate and are kept in a thermostat at 35+-0.1^{0}C for more than half an hour. The kinetic study was followed by mixing thermally equilibrated solution of reactants and transferring the reaction mixture to 1cm^3 cuvette. The progress of reaction was followed by measuring absorbance of the reaction mixture at 410 nm spectrophotometrically using UV-Vis. Spectrophotometer in sulphuric acid medium using water as a reference solvent. The reaction was studied under pseudo-first order condition in which, concentration of hydrazide was in excess as compared to that of ammonium metavanadate.The reaction is found to proceed through formation of complex between vanadium (v) and hydrazide. The pseudo-first order rate constant K was obtained by plotting the log of absorbance at 410 nm against time for hydrazide and was found to be fairly constant at different concentrations of vanadium (v).

2.3 Reaction intermediates:intervention of free radical:

The induced polymerization of acrylonitrile[19] and spontaneous reduction of mercuric chloride[20] in reaction mixture itself indicates that, the present reaction involves the formation of free radical.

2.4 Effect of sulphuric acid on hydrazides:

A standard kinetic experiment was repeated by only changing the concentration of H_2SO_4 from0.0025 to 0.1 M containing equivalentquantities of hydrazides, oxidant, ionic strength, salt and temperature. The mixtures in each flasks were made alkaline and tested for detection of hydrazine after three hours. It was seen that, the resultant solution in various flasks didn't give any response to ammonical $AgNO_3$ and Fehling's solution, indicating absence of hydrazine. Hence it can be concluded that, there is no significant hydrolysis of hydrazide in the presence of H_2SO_4 up to 0.1 M.

2.5 Alternative method for confirmation of status of acid content and to check hydrolysis of hydrazide under experimental conditions:

The confirmation of hydrolysis of hydrazide under experimental conditions was done by conductingsimilar set of experiments. It was subjected to alkalimetric estimation of acid content in the beginning and at the end; when it was found that there was no perceptible change in acid content of any mixture after a period of minimum three hours. This invariance of acid concentration can be attributed to the fact that; there was no hydrolysis of hydrazide under experimental conditions was observed. Similar observations are already reported.[21]

2.6 Determination of stoichiometry of the reaction:

The number of moles of V(v) consumed by one mole of hydrazide in oxidation reaction under investigation means the stoichiometry of the reaction was determined by spectrophotometric method. A set of five reaction mixtures containing a known excess of V(v) over hydrazide in the presence of 1.0 x 10^{-2} M H_2SO_4 and 5.0 x 10^{-2} M $NaClO_4$ was kept in a thermostat at 35^{0}C for more than 48 hours. A blank experiment without hydrazide was carried out concurrently using identical quantities of V(v), H_2SO_4 and $NaClO_4$ after volume correction with water for hydrazide solution.

After completion of the reaction, concentration of V(IV) was determined at 765nm (ε =17.77). [22] from its absorbance and molar extinction coefficient (**ε**) by using the expression C = Abs. / **ε**

Finally, the moles of V(IV) formed in oxidation of one mole of hydrazide were calculated in each case by using the relation. Mole ratio = - [V(IV)] / $[Hydrazide]_0$

The value of [V(IV)] / $[Hydrazide]_0$ for different experiments was found to be nearly equal to 4. Therefore it is concluded that 4 moles of ammonium metavanadate were required for oxidation of one mole of hydrazide.

2.7 Identification of oxidation products:

For the identification of oxidation products; the reaction was studied by using hydrazide and ammonium metavanadate in stoichiometric proportions in presence of 1.0 x 10^{-2} M sulphuric acid and 1.0 x 10^{-1} M sodium per chlorate. The flask containing reaction mixture was kept in a thermostated water bath maintained at 35^{0}C for 24 hours to complete the reaction. After the completion of the reaction, the reaction

mixture was subjected to ether extraction and acid was separated. The presence of carboxylic acid group was detected by testing with 5 percent bicarbonate solution. The amide derivative of the corresponding aromatic carboxylic acid was prepared[23]. The observed physical constant of amide derivatives was found to be in good agreement with those of benzamide. The formation of corresponding carboxylic acids in oxidation of hydrazides was accompanied by evolution of nitrogen gas. Nitrogen was detected by lime test. [24]A mixture of lime and manganese dioxide in 10:1 proportion was ignited in a small hard glass tube. A test portion of concentrated reaction mixture was rendered neutral with sodium hydroxide solution and it was added to the ignited mixture. The tube was heated slowly and the liberated gas was tested with filter paper moistened with manganese nitrate and silver nitrate solutions. This indicator paper held at the mouth of the tube shows grey fleck, which turns blue immediately on treatment with a drop of benzidine solution which, indicates the formation of nitrogen gas during the oxidation. Finally considering the products of oxidation, observed mole ratio and material balance, the oxidation of hydrazide can be represented by the stoichiometric equation

$$Ar.CO\text{-}NH\text{-}NH_2 + 4V(v) + H_2O \xrightarrow[NaClO_4]{H_2SO_4} Ar\text{-}COOH + N_2 + 4\,V(IV) + 4H^+.$$

The above equation not only consistent with stoichiometry and products of reaction, but also it explains the observed gradual decrease in k values with increase in time, which can be attributed to increasing proportions of H^+ ions with the progress of the reaction.

III. RESULTS AND DISCUSSION:

It was found that, the reaction proceeds with a measurable velocity using 5.0 x 10^{-4} M ammonium metavanadate, 5.0 x 10^{-3} M Substrate hydrazide, 1.0 x10^{-1}M salt sodium perchlorate , 1.0 x 10^{-2} M sulphuric acid and 410nm λmax at 35^{0}C. The observed rate constant (k) of the reaction goes on slightly decreasing with time, the order of reaction with respect to V(v) is one.

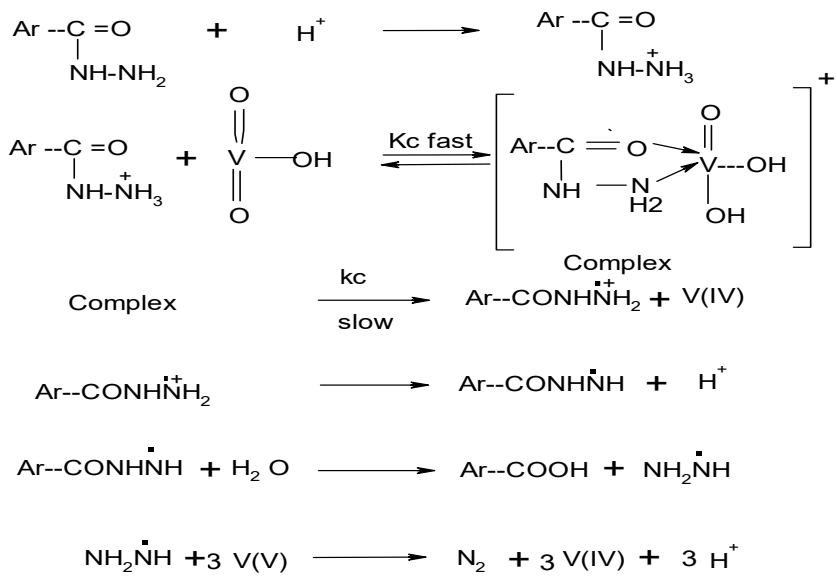
Table 1: The specific reaction rate constants determined by graphical method are:

	Effect of	Unit Concenteration	Multiples of Unit Concenteration and respective rate Constants						
1	[hydrazide]	[p-Cl-BAH]x 10^3M	1.0	2.0	4.0	5.0	6.0	8.0	10
		k x 10^4sec^{-1}	3. 59	2. 69	1. 59	0.989	0.645	0.346	0.189
2	[AMV]	[AMV] x 10^4M.	1.0	2.0	4.0	5.0	6.0	8.0	10.0
		k x 10^4sec^{-1}	1.01	1.03	0.996	0.989	1.02	1.00	0.990
3	[H_2SO_4]	[H_2SO_4] x 10^2M.	0.5	1.0	2.0	3.0	4.0	5.0	--
		k x 10^4sec^{-1}	0.759	0.989	2.01	2.21	2.40	2. 69	--
4	[$NaClO_4$]	[$NaClO_4$] x 10^1M.	0.5	1.0	2.0	3.0	4.0	5.0	--
		k x 10^4sec^{-1}	0.974	0.989	0.984	1.01	0.992	0.996	--
5	various Salts	[SALT]$_o$ =1.0 x10^{-1}M	LiCl	NaCl	KCl	$LiClO_4$	$NaClO_4$	$KClO_4$	$MnCl_2$
		k x 10^4sec^{-1}	1.01	0.997	1.03	0.982	0.989	1.02	1.01
6	Dielectric Constant	Ethanol %	50	60	70	80	--	--	--
		Dielectric constant	64.92	59.98	54. 65	47.78	--	--	--
		k x 10^4sec^{-1}	1.29	0.989	0.797	0.629	--	--	--
7	Temperature	Temperature in ^{o}C	30	35	40	45	50	55	--
		k x10^4 sec^{-1}	0.599	0.989	1.31	2.01	2.46	3.44	--

Table 2: The various Energy parameters calculated are:

Sr	Energy parameters	Value	Sr	Energy parameters	Value
1	Temperature Coefficient	1.89	4	Entropy of activation $\Delta S^{\#}$ (J K^{-1}mol^{-1})	-144.98
2	Energy of activation Ea (KJ mol^{-1})	55.97	5	Free energy of activation $\Delta G^{\#}$ (KJ mol^{-1})	98. 89
3	Enthalpy of activation $\Delta H^{\#}$ (KJ mol^{-1})	45.92			

Mechanism of the reaction:The detailed mechanism of the reaction in terms of the active species of the HVO_3 and substrate protonated hydrazide is shown in the scheme as follows.



where, Ar = C_6H_4CI

According to the above scheme the rate of the reaction is given by

Rate = k_c [Complex]

Substituting the value of [Complex] from the equilibrium,

Rate = k_cK_c [HVO_3] [Ar-CONHNH$_3$] .

SCHEME

IV. CONCLUSION:

The investigation of the effect of hydrazide concentration on the reaction rate shows that, The pseudo-first order rate constant decreases with increase in hydrazide conc. The decrease in rate constant as the concentration of hydrazide increases can be attributed to greater stability of the complex in alcoholic medium probably due to solvation[25] .

The effect of oxidant concentration on the reaction shows that, the rates of oxidation of hydrazide under examination are almost constant with increase in concentration of V(v) and the order of reaction with respect to vanadium (V) remains one throughout the used concentrations of vanadium (v). In other words, Oxidation follows the first order kinetics because the log (Abs) versus time plot in each case is linear with positive slope and intercept on log (Abs) axis. The constancy of k values at different initial [V(v)] indicate the pseudo-first order kinetic behavior of reaction.

The effect of concentration of sulphuric acid on the rates of oxidation of hydrazide under study shows that, the specific reaction rate increases as the concentration of acid increases.

The reaction rates are not influenced by increase in ionic strength similarly the reactions rates are not influenced by using various salts, under the experimental conditions .

The specific rate of reactions is decreasing with decrease in dielectric constant[26].

The study of effect of temperature on oxidation of hydrazide clearly shows that, rate of reaction depends on temperature and it increases with increase in temperature. The values of observed rate constants were used to determine various thermodynamic parameters like temperature coefficient(189), energy of activation (Ea) 55.97 KJ mol^{-1} , enthalpy of activation (ΔH #)45.92 KJ mol^{-1}, entropy of activation (ΔS #)-144.98 J K^{-1} mol^{-1} and free energy of activation (ΔG #)98. 89 KJ mol^{-1}. The log (Abs) against time plots at different temperatures are linear which reveals that, the pseudo-first order kinetic behavior of the reaction is not affected by change in temperature. The formation of free radicals or radical ions during the course of reaction was confirmed from induced polymerisation of acrylonitrile[19, 20]. The formation of carboxylic acids and N2 in the oxidation of aliphatic as well as aromaticacid hydrazides[28] is well documented in chemical literature. The mole ratio of hydrazide:vanadium (v) is found to be 1:4 and it is independent of concentration of sulphuric acid that was used.

The integer value of observed mole ratio, its independence on sulphuric acid concentration and formation of only carboxylic acid along with nitrogen gas as oxidation products leads to deduce that, the two rate determining steps occurring simultaneously results in the formation of one and the same intermediate. Although the observed mole ratio (substrate : oxidant) of the reaction is 1:4, as is pointed out earlier, the order of reaction with respect to vanadium (v) is one.

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Dihydrazone based colorimetric chemosensor for selective determination of Cd^{2+} and pH in mixed aqueous-organic media

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ABSTRACT: *A naked-eye colorimetric chemosensor DAP has been developed and synthesized by simple one pot condensation reaction for selective recognition of Cd2+ ion over other competitive ions in mixed aqueous-organic media. The designed chemosensor displayed a visual color change from pale yellow to orange and remarkable changes in its absorption spectra upon interaction with Cd2+. The binding constant (Ka) and 1:1 stoichiometry of chemosensor towards Cd2+ was determined by the Benesi–Hildebrand (B–H) and Job's plot method, respectively with a detection limit 4.81 μM. The sensing ability of DAP for Cd2+ was further confirmed by DFT studies and the proposed chemosensor was successfully used for the determination of Cd2+ ions in water samples. Moreover, the resulting DAP-Cd2+complex were further used as a colorimetric pH indicator based on color change due to DAP-Cd2+ complex.*

Keywords: *Colorimetric chemosensor, Cd2+, DFT*

I. Introduction

Recently, design and synthesis of colorimetric chemosensor for selective detection of heavy transition metal ions receiving a great deal interest by researchers because these metal ions show a crucial role in biological, environmental and chemical processes [1-3]. Among the several transition metal ions cadmium is a soft, silver-white trace and toxic metal occurs in the earth crusts. Cadmium is widely used in large number of industrial and agriculture applications such as rechargeable Ni-Cd batteries, stabilizers for alloys, electroplating, semiconductors, dyes and pigments, cement industries, phosphate fertilizers, leads to discharge of cadmium during such activities into the soil, water and air, causes diverse effect on ecological system[4-6]. In human cadmium and its complexes are accumulated in tissues through ingestion of contaminated food and water [7]. The long-term exposure of Cd^{2+} in human causes anemia, diarrhea, renal dysfunction, decalcification, reduced lung capacity, changes in vitamin-D metabolism, kidney damage, hypertension and increase in risk of lung, kidney, breast and prostate cancer [8, 9]. Due to toxic nature of cadmium the USEPA listed it is one of the primary pollutants. Also, WHO (world health organization) and USEPA underlines the permissible level of Cd^{2+} in drinking water is 3 ppb and 5 ppb respectively [10-12]. Therefore, it is importance to develop highly selective and sensitive colorimetric chemosensors for recognition of Cd^{2+} in environmental and biological system.

To date many conventional sensing methods for the detection of Cd^{2+} employed [13-15] which are powerful and sufficient for the detection of Cd^{2+} however, these methods are tedious, time consuming and required use of expensive, sophisticated instrumentation. Compared to this conventional analytical methods, colorimetric methods gives more attention because this method requires simple experimental procedure, inexpensive instrumentation and can easily detects analyte with naked eyes in visible light range with high selectivity [16, 17]. Up to now, considerable works have been made concerning to development of colorimetric and fluorescent chemosensors for detection of Cd^{2+} ions; however most of the chemosensor work in organic solvent which limits practical applications of chemosensor inenvironmental and biological system [18, 19]. Furthermore, chemosensors for recognition of Cd^{2+} in aqueous medium are still scarce. Thus, there is a great need to develop sensor that works in aqueous medium [17, 20].

At the same time, development of chemosensor that are able to determine pH has presently been evolving as a prime research area as it plays vital roles in many biological and chemical processes [21, 22]. In recent years numbers of fluorescent and colorimetric pH chemosensors via different signaling mechanism [23-25] have been reported with advantages such as high sensitivity, real-time visualization and easy operation over the traditional pH glass electrodes [26] but most of reported colorimetric pH sensors have limitations such as complicated synthetic procedures, weaker pH sensitivity than fluorescent probes [27] and are still scarce [28, 29]. Therefore, development of a highly pH sensitive colorimetric chemosensor showing significant color is of great interest in physiological conditions.

In this context, herein we reported a simple colorimetric chemosensorDAP based on the

combination of the hydrazone moiety and the dimethyl-aniline one for the recognition of Cd^{2+} in in aqueous medium over the other coexisting ions and successfully used for environmental monitoring of Cd^{2+} in water sample and determine pH in certain range by change in color of receptor DAP in the presence of Cd^{2+} ions.

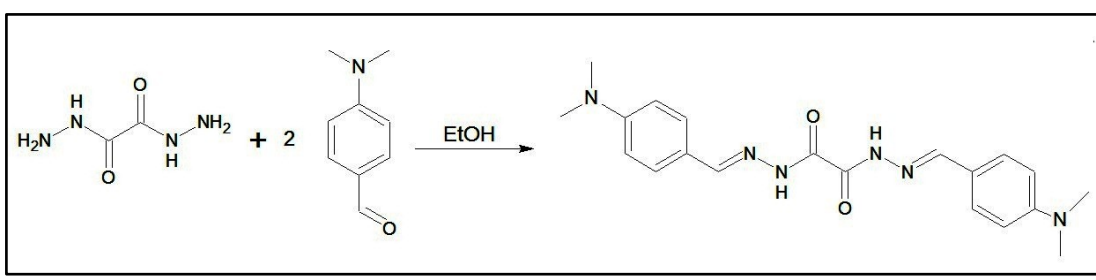
II. Experimental:-

2.1. Materials and measurements:-

Oxalic acid dihydrazide, 4-(dimethylamino) benzaldehyde, ethanolwere purchased from R. L. fineChem Industries, Mumbai, India and used without further purification. Nitrate and sulphate salts of different cations were purchased from S. D. Fine-Chem. Ltd. (Mumbai, India). All analytical grade solvents were purchased from available commercial sources and were used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AC 300 MHz NMR spectrometer using DMSO-d_6 as solvent and TMS as internal standard. The elemental analyses were performed on a Vetro-ELIII elemental analyzer. UV-Vis absorption spectra were recorded on ELICO SL-210 UV-Vis double beam spectrophotometer at room temperature using a quartz cell and pH values were measured by using digital pH-meter (Equip-Tronics EQ-614 A).

2.2. Synthesis of chemosensor:-

The sensor DAP was synthesized by condensation of 4-(dimethylamino) benzaldehyde (2 mmol) and oxalic acid dihydrazide (1 mmol) in absolute ethanol under acidic medium for about 12 hours at room temperature. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate, 7:3, v/v). After the completion of reaction, product was filtered, washed with water and recrystallized from ethanol to give yellow solid.



Scheme 1. Synthesis of Chemosensor**DAP**.

Pale yellow solid, Yield: 83%, m.p. > 300 *°C*; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.93 (s, 2H, NH); 8.22(s, 2H, CH); 7.54-6.69 (m, 8H, Ar–H); 2.91 (s, 12H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 39.27, 111.59, 112.03, 121.38, 128.99, 129.29, 149.38, 152.08, 156.18 Anal. Calc. for $C_{20}H_{24}N_6O_2$: C, 63.14; H, 6.36; N, 22.09. Found: C, 63.47; H, 6.14; N, 21.97.

2.3. Experimental procedure:

Stock solution of sensor (DAP**)** was prepared at 1x 10^{-4} M in Bis-tris buffer: MeCN (8:2, v/v, pH = 7.0) and stock solution of salts of different cations (2 x 10^{-3} M) was prepared in bis-tris buffer (pH = 7.0) using double distilled water. For UV-Vis spectral measurements solution of DAP was further diluted to 5 ml to give final concentration 10 μM and then absorption spectra was recorded by adding 10 equiv. of each cations to the solution of DAP.

III. Result and discussion:

3.1 UV-Vis studies of DAP toward metal ion:

The UV–Vis and colorimetric sensing studies of chemosensorDAP for recognition of cation were carried out in presence of different biologically important metal ions (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Al^{3+}, Pb^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+}, Hg^{2+}) by using UV-Vis absorption spectroscopic techniques in Bis-tris buffer: MeCN (8:2, v/v, pH=7.0) media. **Figure 1** shows the UV-Vis absorption spectra of free DAP exhibited maximal absorbance at 396 nm. Upon the addition of 10 equiv. of different metal ions into the solution of DAP **(**10 μM), little or almost no changes occur in the absorption peak of DAP. However, in presence of Cd^{2+} ions spectral changes occurs in the absorbance spectrum of DAP with bathochromic shift of absorption band and showed a naked eye color change from yellow to orange.

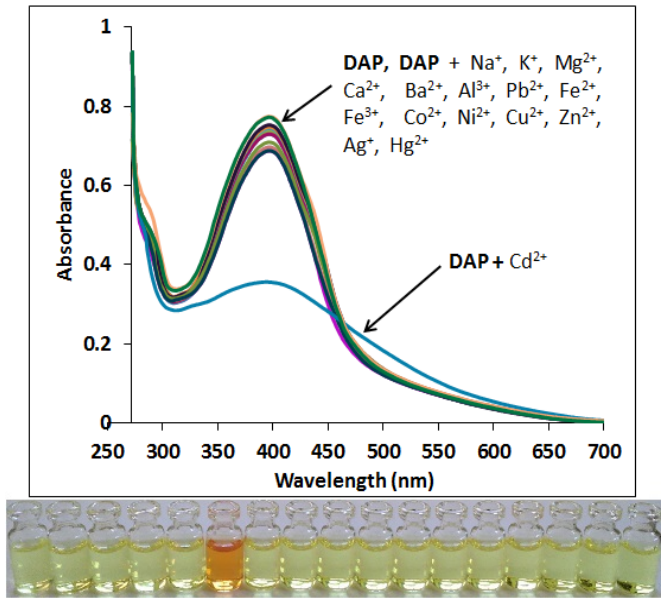


Figure 1. (a) UV-Vis spectra of DAP (10 μM) in presence of different metal ions (10 equiv.) in Bis-tris buffer: MeCN (8:2, v/v, pH=7.0) solution and (b) corresponding color changes.

To further examine the binding affinity of DAP with Cd^{2+} UV-Vis titration experiment were conducted with different concentration of Cd^{2+} at pH= 7.0 using bis-tris buffer.**Figure 2a** shows upon gradual addition Cd^{2+} into solution of DAP, the absorption peak decreases gradually at 396 nm and a new red shift absorption peak emerges at 500 nm. Meanwhile, isosbestic point observed at 463 nm indicating a clear conversion of chemosensor into a single stable species of sensor and Cd^{2+} and the binding mechanism may be due to the ligand to metal charge transfer (LMCT), which is responsible for color change from yellow to orange upon binding of DAP with Cd^{2+}. To further verify DAP as a colorimetric chemosensor for Cd^{2+}competitive experiment was carried out in presence of other competitive cations. For competitive experiment,sensor DAP (10 μM) was treated with 10 equiv. of Cd^{2+} in presence of 10 equiv. of other cations. The result shows that competitive cations did not induce significant changes in theabsorbance in comparison with that observed in thepresence of Cd^{2+} alone to the DAP in solution (**Figure2b)**. Thus, chemosensor displays excellent selectivity for Cd^{2+} over other tested competitive cations with notable color change from yellow to orange.

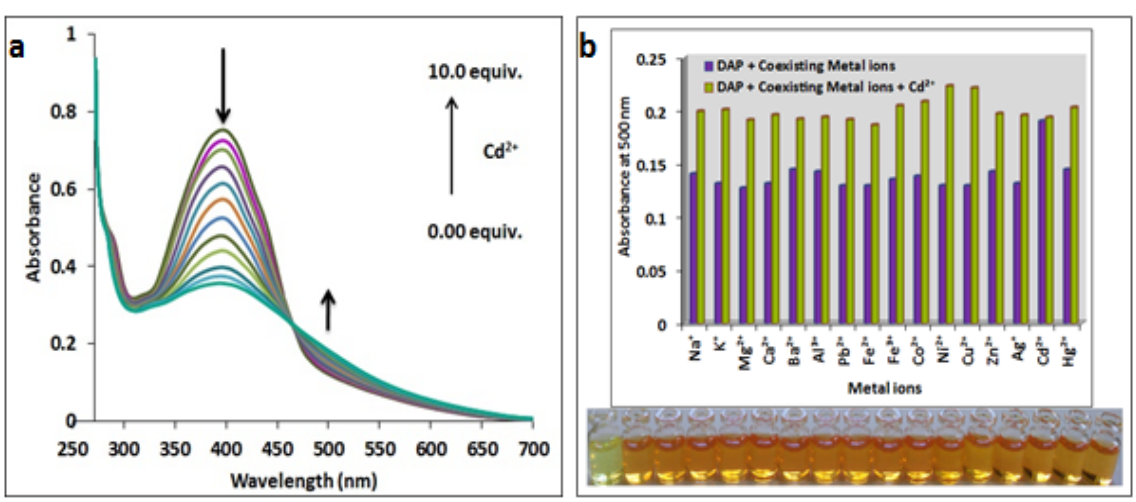


Figure 2a. UV–visible spectral changes of DAP (10 μM) with increasing concentrations of Cd^{2+} in Bis-tris buffer: MeCN (8:2, v/v, pH=7.0) solution.

Figure 2b. (a)absorbance responses of DAP in theabsence and presence of Cd^{2+} ions (10 equiv.) and several competing metal ions(10 equiv.) in Bis-tris buffer: MeCN (8:2, v/v, pH=7.0) solution at wavelength 500 nm.

The violet bars represent the absorbance response of DAP in the presence of competing metal ions (equiv.). The green bars represent the absorbance response of DAP upon the addition of competing metal ions +Cd^{2+} and corresponding visible color changes of DAP (10 μM) in the presence of Cd^{2+} (10 equiv.) and other competitive metal ions (10 equiv.).

IV. Calibration Curve, LOD, stoichiometry and binding constant of Cd2+ complex:

Under the optimized condition, the absorption spectra of DAP with different concentration of Cd^{2+} were recorded. The experimental data for Cd^{2+} were plotted to obtain a linear relationship. Here, sensor gives good linearity in the calibration graph ($A-A_0$) as a function of Cd^{2+} concentrations at wavelength 396 nm in the range 0.5 to 9.0 equiv. with correlation coefficient 0.9974 (**Figure 3a**) and further the addition of Cd^{2+} in DAP, no further variation are observed in the absorption spectra indicating that sensor reached saturable concentration of Cd^{2+}. Importantly, the plot of ($A-A_0$) against concentration of Cd^{2+} fit a linear Beer-Lambert equation.

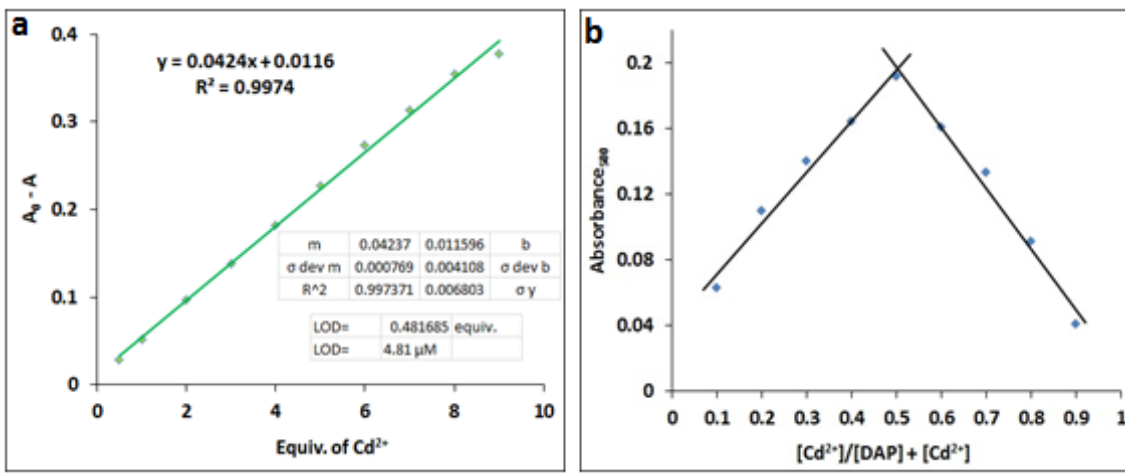


Figure 3a. Calibration plot of the A_0–A versus concentration of Cd^{2+} shows that system is linear in between 0.5 to 9.0 equiv. with DAP (10 μM) at 396 nm.

Figure 3b. Job's plot showing 1:1 complex formed between chemosensor DAP and Cd^{2+} inBis-tris buffer: MeCN (8:2, v/v) solution.

The limit of detection was calculated by equation, LOD=$\frac{3\sigma}{k}$

Where, σ is the standard deviation of the y-intercept of regression line and *k* is the slope of calibration curve [30]. Here, LOD of sensor for Cd^{2+} is 4.81 μM. The stoichiometry of DAP and Cd^{2+} ion complex was determined by Job's method [31] by keeping the sum of the concentration of Cd^{2+} and sensor constant and varying the mole fraction from 0.1 to 0.9. The Job's plot reached maximum at 0.5 confirming 1:1 stoichiometry complex between sensor and Cd^{+} (**Figure 3b**). Furthermore, binding constant was determined by Benesi-Hildebrand equation [32] and it was found to be, Ka= 4.8775 x 10^3 M^{-1}.

V. Effect of pH:

Since for the practical application of chemosensor, the proper pH value of the solutionis of great importance in the detection procedure as it affects sensor spectral properties. The effect of pH on absorbance spectra of DAP and its Cd^{2+} complex were investigated in Bis-tris buffer: MeCN(8:2, v/v) in the pH values ranging from 2.0-12.0. **Figure 4** indicates that free receptor DAPshowed no obvious changes in the absorption spectrum. While, in presence Cd^{2+} ions increase in absorbance of Cd^{2+}-DAP complex was observed in the pH range 6.0 to 12.0 with change in color from yellow to orange. These results showed that Cd^{2+} could beclearly detected by UV-Vis absorption spectral measurement or by the naked eyeover a wide pH range.

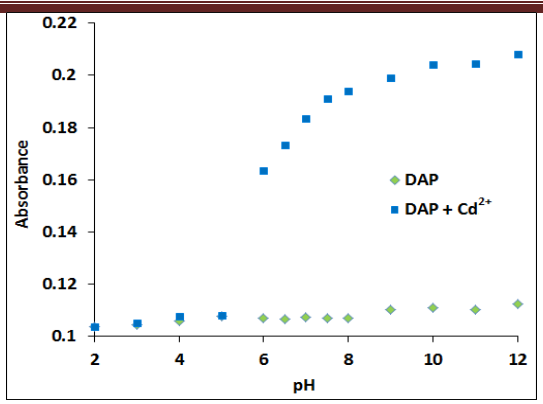


Figure 4. Effect of pH on the absorbance of DAP (10 μM) in absence and presence of Cd^{2+}.

VI. DFT studies:

To better understand the coordination of DAP with Cd^{2+} density functional calculations (DFT) calculation performed by using ORCA program package (version 3.0.3, developed by Prof. Dr. Frank Neese). The geometries of DAP and DAP-Cd^{2+} were optimized by using B3LYP/def2-TZVP and B3LYP/def2-SVP with effective core potential (ECP) based basis set respectively [33]. The energy-minimized structure of DAP and DAP-Cd^{2+} were shown in **Figure 5a**. The energy minimized structure of sensor showed a planar structure with dihedral angle = −179.815° (1O, 2C, 3C, 4O). Furthermore, the DAP-Cd^{2+} complex showed Cd^{2+} was coordinated with 5N, 6N atoms of sensor and two oxygen atoms of H_2O, and exhibited a tetrahedral structure with dihedral angle = −0.109° (1O, 2C, 3C, 4O). The frontier molecular orbitals of DAP and DAP-Cd^{2+} complex are shown in **Figure 5b.**The energy band gap (ΔE) between HOMO and LUMO for DAP and DAP-Cd^{2+} were found to be 4.662 eV and 2.631 eV respectively. The lowering of HOMO–LUMO energy gap upon binding of sensor with Cd^{2+} is due to the ligand-to-metal charge-transfer (LMCT), which results in a new charge transfer absorption band observed at 500 nm in the UV-Visible spectra with solution color change from yellow to orange. Hence, based on the above results we propose the proposed binding mode of DAP for Cd^{2+} is as shown in **Scheme 2**.

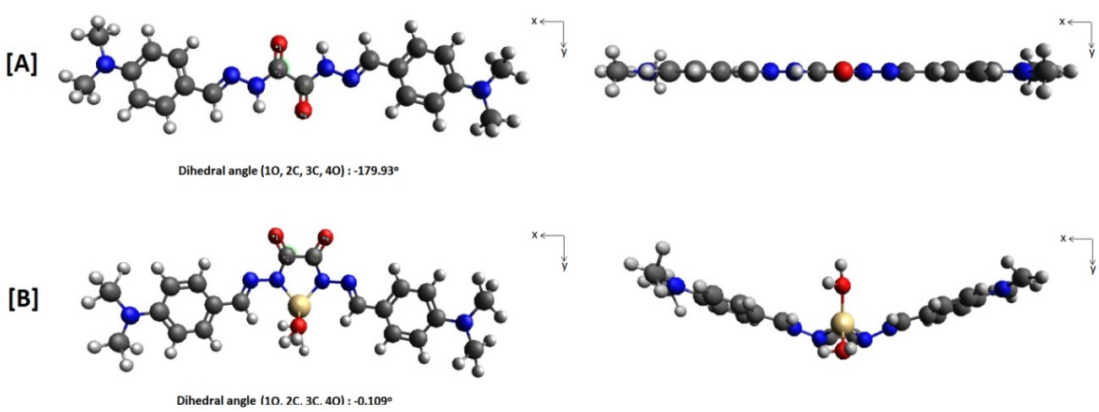


Figure 5a. The energy-minimized structures of (A) DAP and (B) DAP-Cd^{2+} complex.

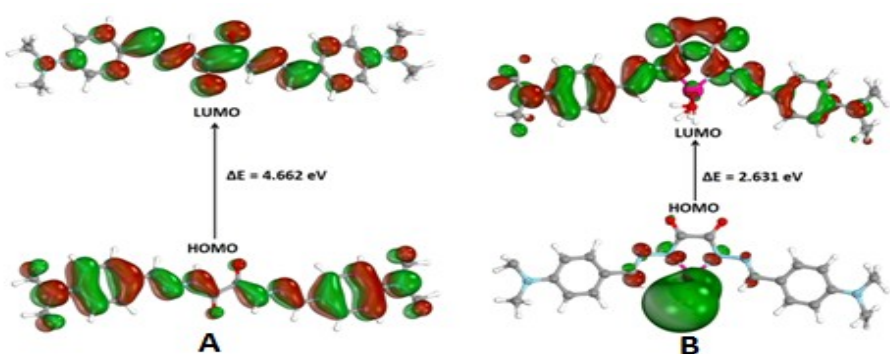
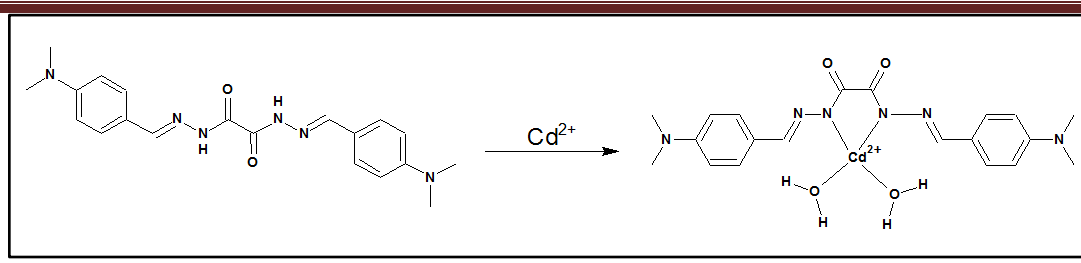


Figure 5b. HOMO and LUMO energy level of (A) DAP and (B) DAP-Cd^{2+}.



Scheme 2. The proposed binding mode of chemosensorDAP for Cd^{2+}.

VII. Secondary sensor behavior of DAP-Cd2+ complex as pH indicator:

As expected, preliminary test validated that the addition of Cd^{2+} ions induces a significant color change from yellow to orange in the pH range 6.0 to 12.0; a systematic study was performed so that the DAP–Cd^{2+} complex may act as a secondary sensor. Hence, pH titration experiment were conducted to determine the exact pH value at which color of solution turns yellow to orange by preparing the solution of DAP in presence of Cd^{2+} in the pH range 5.1- 5.9 (**Figure 6**). From the experiment it was observed that the color of solution changes after the pH 5.6; indicates above pH 5.6 due to the formation of the stable DAP-Cd^{2+} complex the solution becomes orange while below pH 5.6 the protonation ofhydrazone -NH moiety of DAP inhibits the co-ordination of Cd^{2+} to DAP (**Scheme 3**). Thus we can easily determinewhether the pH value of certain solution is below or above 5.6 by naked eye color change in the presence of DAP-Cd^{2+} complex. Likewise, **Figure 6** shows with increase in pH from 5.1- 6.0 the absorption peak of DAP-Cd^{2+} complex decreases sharply at 396 nm and a new absorption band appears at 487 nm with isosbestic point at 451 nm indicates that an equilibrium occurs betweenDAP and their protonated/deprotonated forms and the occurrence of new absorption bands at 487 nm of DPA-Cd^{2+} complex with increasing pH may be due to deprotonation of hydrazone (NH) protons which induce MLCT process across the hydrazone moiety.

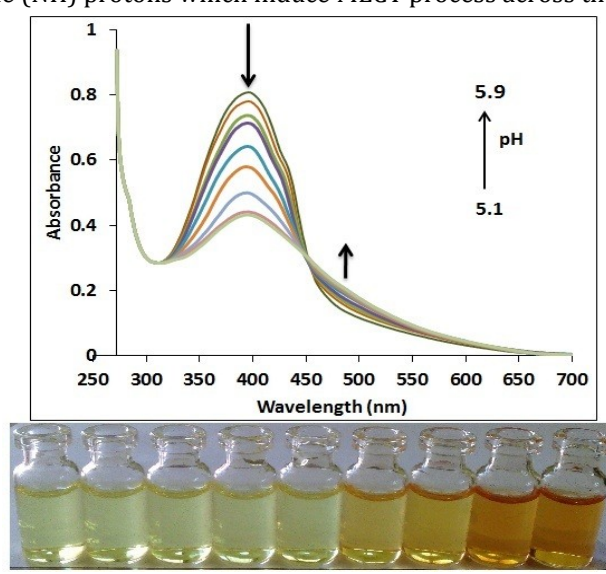
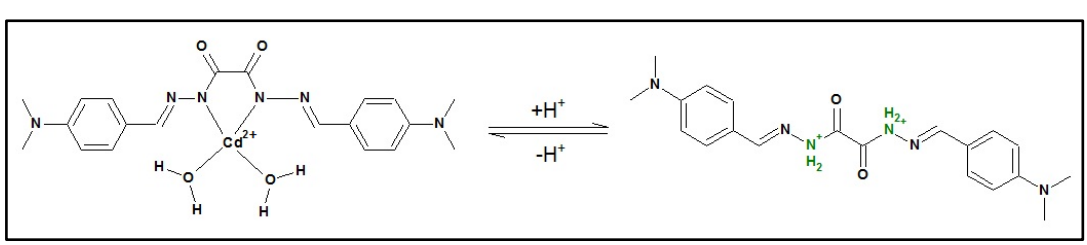


Figure 6. The UV-Vis spectral and color changes of the DAP-Cd^{2+} complex at different pH values (5.1 to 5.9) in Bis-tris buffer: MeCN (8:2, v/v) solution.



Scheme 3. Plausible pH response mechanism by protonation and deprotonation of the DAP.

VIII. Applications:

From the experimental data it was observed that, DAP was colorimetric sensor for Cd^{2+} with high selectivity and sensitivity we explored the utility of chemosensor for the determination of Cd^{2+} in the environmental water sample as well as DAP-Cd^{2+} complex as a pH indicator.

8.1. Water sample analysis:

Water samples were collected from local region of campus. Before analysis collected water samples were first filtered through Whatman filter paper No. 41 to remove insoluble impurities and analyzed directly under suitable condition by standard addition method within linear working range at two different concentration levels. The results are listed in **Table 1**which shows that, DAPis able to measure the concentrations of spiked Cd^{2+} in good agreement with average recovery of the spiked sample, which in turn confirmed the consistency and practicality of this method.

Table 1 Determination of Cd^{2+} in water samples via standard addition method (n=3)

Water sample studied	Amount of Standard Cd^{2+} Added ($\mu g\ mL^{-1}$)	Total Cd^{2+} found (n=3) ($\mu g\ mL^{-1}$)	Recovery of Cd^{2+} added (n=3) (%)	RSD (%)	Relative Error (%)
River Water *	15.0	15.12	100.82	4.17	0.80
	45.0	45.73	101.62	0.85	1.62
Industrial waste Water**	15.0	15.71	104.73	4.84	4.73
	45.0	46.14	102.53	2.86	2.53
*Hiranyakeshi River, Gadhinglaj, (MS), India, **MIDC Gadhinglaj, India					

8.2. pH indicator:

As the color of the DAP-Cd^{2+} complex were changes from yellow to orange above pH 5.6, we tested the DAP-Cd^{2+} complex as a pH indicator in real environmental samples. Therefore we conducted colorimetric experiment with samples of soft drink and tap water to determine their approximate value of pH. Upon addition of tested sample in the solution DAP-Cd^{2+} complex, the color of tap water changes from yellow to orange while the color of soft drink remains yellow, indicates the pH of soft drink sample is lower than 5.6 (**Figure 7**). Hence the above complex might be used as a pH indicator in environmental analysis.

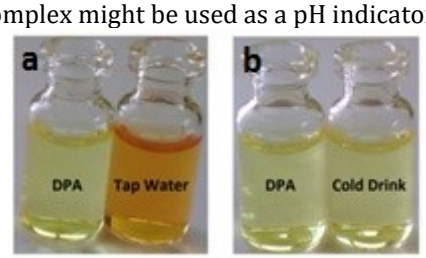


Figure 7. Color changes of (a) tap water and (b) Soft Drink sample in presence of the DAP-Cd^{2+} complex (10 μM).

IX. Conclusions:

In summary, herein we report, a hydrazone based colorimetric chemosensor DPA for the detection of Cd^{2+} ions over other coexisting cationsin aqueous-organic medium. Chemosensor DPA showed high sensitivity and selectivity towards Cd^{2+} ion in a 1:1 stoichiometric proportion, which induces spectral changes in absorption spectra based on a LMCT mechanism. DFT studies also support the structural and optical properties of DPA and DPA-Cd^{2+}. Importantly this chemosensor was successfully used to quantify Cd^{2+} in water samples. Moreover, the DPA-Cd^{2+} complex were used as a colorimetric pH indicator.

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Studies on the Physico Chemical Parameters of Soil from Northern Part of Shrirampur Tehsil Area, District Ahmednagar, (M. S.) India

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ABSTRACT: *Soil is a natural body of mineral and organic material differentiated into horizons, which differ among themselves as well as from underlying materials in their morphology, physical make-up, chemical composition and biological characteristics. In Shrirampur area due to industrialization and other anthropogenic activity the soil from its northern part has been polluted. The The sewage water in the Mula River flows through the Belapur village and hence it is felt necessary to carry out the soil analysis to understand the pollution levels of the soils in the adjoining area. Since plants depend on the soils for their nutrients, water and minerals supply, the soil type is a major factor in determining what types of plants will grow in an any area. In the present study the analyses of soil samples collected from the sugarcane field of Belapur, Devlali, and Narasari located in the Shrirampur area which is influenced by the solid waste disposal as well as industrial effluents. In the first place soils samples from 12 representative locations were collected for their analysis. Physical parameters like pH, Electrical conductivity (EC), organic carbon (%) and chemical parameters like phosphorus, potassium, copper, iron, manganese, zinc, Calcium and boron were analyzed. From this study it has been revealed that there is excessive dose of phosphorous and potassium into the soil because most farmers are using excessive chemical fertilizers. Similarly Cu, Fe, Mn and Zn concentration has also been seen higher than the normal range and due to poorer drainage conditions of this area making soil alkaline. Thus it is concluded that variable concentrations of various parameters and irregular distributions of micronutrients may be attributed due to the added fertilizers during the crop formation.*

Keywords: *Anthropogenic contamination, Micronutrients, Soil analysis, Shrirampur, Major Constituents.*

I. Introduction

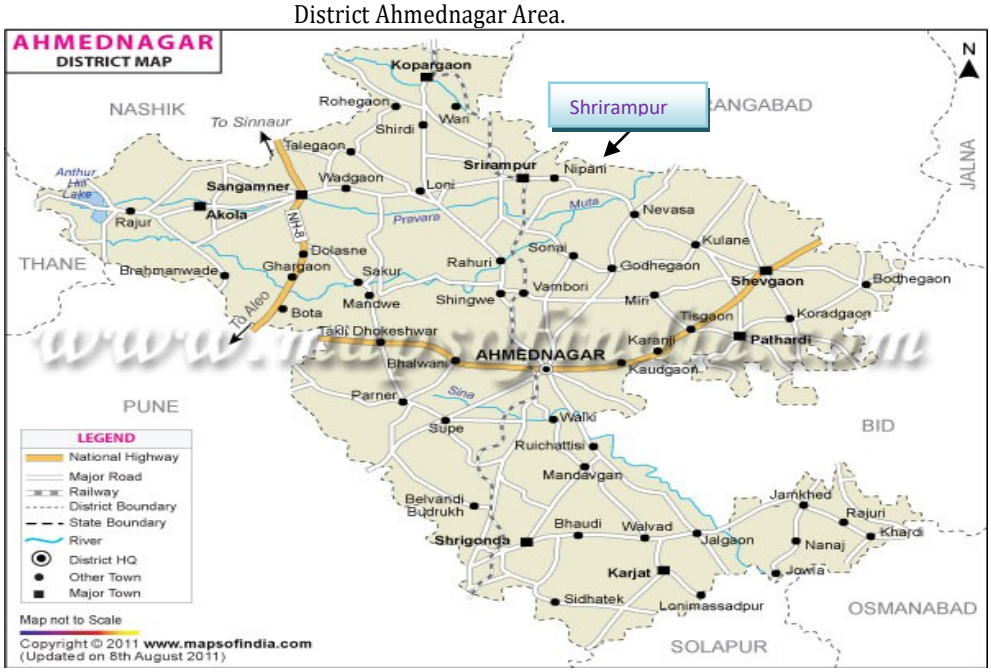
In Shrirampur due to average industrialization and other anthropogenic activities the soil from northern part of city gets polluted. The Mula river carries sewage water of Shrirampur area through from Belapur village and hence it is more relevant to carry out soil analysis. Soil is an unconsolidated material of the earth's crust in which terrestrial plants grow if water and temperature are adequate with minimum available nutrients. According to [11] the soil is a natural body of mineral and organic material differentiated into horizons, which differ among themselves as well as from underlying materials in their morphology, physical make-up, chemical composition and biological characteristics [18]. Soil can develop from weathered rocks, volcanic ash deposits or accumulated plant residues. Soil thus form a substrate for plant growth which performs many functions essential to life and in general, most plants grow by absorbing nutrients from the soil whose ability to do this depends on the nature of the soil. Soil formation is a constructive as well as destructive process [15] the predominant destructive process are physical and chemical breaking down of materials, plants and animal structures which result in the partial loss of more soluble and volatile products. Soil types are a major factor in determining what types of plants will grow in a certain area as plants use inorganic elements from the soil such as nitrogen, potassium and phosphorus. However microorganisms like fungi, bacteria and other microscopic life forms available within the soil are also vital and hence soil is a dynamic medium made up of minerals, organic matter, water, air and microorganisms. The nature of soil primarily depend upon its continued change under the effect of physical factors like the parent material, time, the climate, the organic activity in it etc.[18]. Since soil is made up of such diverse materials like weathered rock particles and organic material (humus), it can be classified into various types based on the size of the particles it contain [19, 5]. The modern concept of soil quality is the ability to sustain plant and animal productivity, to increase water and air quality and to contribute plant and animal health [3, 4]. Although all physico-chemical properties are involved in soil functioning, bio chemical properties tend to react most rapidly to get change in the external environment [13, 2]. The objective of this study is to 1) to analyze the soil samples of Belapur and Narsari to investigate the effects of anthropogenic activity on the crop productivity, 2) to study the effect of sewage water of Shrirampur area carried by Mula river through Belapur village on the soils, 3) to evaluate the effects of excessive use of chemical fertilizers by the farmers on the characteristic of soils in Belapur and Narsari.

II. Materials and Methods:

2.1Study area:

The geographical area of Shrirampur tehsil is 04 Sq. Km. which is about 2.4 per cent of the state's total geographical area. It lies between 19.6195° North latitude and 74.660° Eastern longitude. The present study was conducted jointly in drought prone zone, Shrirampur Tehsil, where agriculture farming and related production is one of the major sources of wage earning. Four villages were randomly selected from the study area for the quantification of soil composition. As this region of Shrirampur tehsil falls in irrigation area, the selected soil samples were analyzed for their physical and chemical composition with the help of standard techniques of analysis. Data so obtained were correlated with the help of standard equations and graphs showing their chemical variation from village to village [25].

Figure:a) Location Map of and Soil Samples Collected Villages Belapur and Narsari from Shrirampur Tehsil District Ahmednagar Area.



2.2 Methods:

The present study deals with the analysis of soil samples from sugarcane field which were collected in a period 2016 - 2017 from Belapur and Narsari villages situated towards of Shrirampur area and this region is affected by the solid waste disposal as well as industrial effluents. This study was primarily focused on testing of soil quality from 20 representative sampling stations (numbered as 1 to 20) and the analytical results were expected to be representative for the entire field. The surface contaminated soil material were removed using spade or khurpi [7] and for sampling V shaped holes were dug for collecting a uniform 2 cm thick slice of soil up to a depth of 22cm. which were collected in a Cotton bags. Samples collected were thoroughly mixed on a piece of clean cloth, air dried and the lumps were broken using wooden pestle and mortar [20]. Particles were disaggregated, crushed and sieved with 20mm mesh diameter, stored in glass bottles and labeled.

pH values were determined using Equiptronics pH meter as described by [10]. For this 10 g soil sample was mixed with 30 ml distilled water in 1: 3 ratio. The suspension was stirred intermittently with glass rod for 30 minutes and left for one hour. The combine electrode was inserted into supernatant and pH was recorded. pH value as a measure of the hydrogen ion activity of the soil water system and expresses the acidity and alkalinity of the soil. It is a very important property of soil as it determines the availability of nutrients, microbial activity and physical condition of soil.

Electrical conductivity (EC) expresses ion contents of solution which determine the current carrying capacity thus giving a clear idea of the soluble salts present in the soil. The electrical conductivity of a soil samples was determined on an Equiptronics digital electrical conductivity bridge for which 10g soil was added in 30ml distilled water. The suspension was stirred intermittently for half an hour and kept it for

30 minutes without any disturbances for complete dissolution of soluble salts. The soil was allowed to settle down and then conductivity cell was inserted in solution to take the reading to record the EC values.

Organic matter is useful in supplying nutrients and water to the plants and also provides good physical conditions to the plants. The quantity of organic carbon in the soil was estimated by using modified Walkey- black method [23] as described by [10]. 1g finely ground dry soil sample was passed through 0.5mm sieve without loss and was taken into 500ml conical flask. To this 10ml of 1N potassium dichromate and 20ml con. H_2SO_4 were added and the contents were shaken for a minute and allowed to set aside for exactly for 30 minutes and then 200ml distilled water, 10ml phosphoric acid and 1ml diphenylamine indicator were added. The solution was titrated against standard ferrous ammonium sulphate till colour changes from blue violet to green. The blank titration was also carried without soil.

In soils available phosphorus is found as orthophosphate in several forms and combinations but only a small fraction of it may be available to plants. Available phosphorus was estimated by Olsen's method [14] modified by [24]. The reagent for Olsen's P was 0.5 M $NaHCO_3$ (pH 8.5) prepared by dissolving 42g $NaHCO_3$ in distilled water and made up to 1 lit. The pH was adjusted at 8.5 with 20% NaOH solution. 2.5g of air dried soil was weighed into 150ml Erlenmeyer flask, 50ml of Olsen's reagent (0.5 M $NaHCO_3$ Solution, pH 8.5) and one teaspoonful of activate charcoal were added. The flasks were shaken for 30 minutes and contents were filtered immediately through Whatmann filter paper No. 41. 5ml of the filtrate was taken out by pipette into 25ml of volumetric flask and was neutralized with 1: 4 H2SO4 using p-nitrophenol as indicator and the volume was made up by adding distilled water. After addition of few crystals of stannous oxalate blue colour developed and intensity of blue colour was read in photoelectric colorimeter within 10 minutes at a wavelength of 730nm. A blank was run without soil. Potassium in soil water has been estimated by flame by preparing the standard solutions of potassium (ppm) and feeding the diluted extract in flame photometer for recording the reading for standard and sample with K filter.

Micronutrients like Cu, Zn, Fe, and Mn are estimated by using Atomic Absorption Spectrophotometer employing standard methods [22]. Micronutrients include Iron, Manganese, Zinc, Copper, and Boron. The term refers to plant's needs, not to their abundance in soil. They are required in very small amounts but are essential to plant health in that most are required to speed up plant's metabolisms. They are generally available in the mineral component of the soil and the method commonly used for determination of available micronutrients in soil samples is by [12] This method consists of use of DTPA (Diethylenetriaminepentaacetic acid) as an extractant which has been widely accepted for the simultaneous extraction of micronutrients like Zn, Cu, Fe Mn in neutral and alkaline soils. Most commonly used method for available boron is hot water extraction method as given by [1] which has been modified by [8] in which boiling the soil with water is employed. The extracted boron in the filtered extract is determined by azomethine-H colorimetric method.

III. Result and Discussion:

3.1. Belapur Area from Shrirampur Tehsil District Ahmednagar:

An examination of soil samples (Table 1) shows that the values for pH range from 7.32 to 8.52 (Fig.b) indicating that the soils are alkaline and under such conditions the solubility of minerals decreases creating nutrient deficiencies in the soils. Plant growth is therefore limited by deficiencies in iron, manganese, zinc, copper and boron. Electrical Conductivity value ranges from 0.20 mS/cm to 3.02 mS/cm (Table 1), however sample No. 6 shows excess content of soluble salts which may due to excess use of fertilizer like K. Electrical conductivity is used to estimate the soluble salt concentrations in soil and is commonly used as a measure of salinity. Soil with EC below 0.4mS/cm is considered marginally or non-saline, while soils above 0.8 mS/cm are considered severely saline. The soils under analysis were found moderately saline except sample Nos. 4, 7, and 10 (Fig. b).

The organic carbon (%) ranges from 0.38 to 1.5 % (Fig.b).The organic soil matter includes all the dead plant materials and live or dead animals. Most living things in soils, including plants, insects, bacteria and fungi, are dependent on organic matter for nutrients and energy. Soils have varying organic compounds in varying degrees of decomposition. Organic matter holds soils open, allowing the infiltration of air and water, and may hold as much as twice its weight in water. Phosphorus is one of the key macronutrient required for plant growth and metabolism. Inorganic phosphate supplied to the soil as a fertilizer is rapidly converted into unavailable form. Soluble P converted into insoluble phosphate involves microorganisms. Phosphorous in the present soils vary from 101.1Kg/hectare to 340.0 Kg/hectare (Fig. b) the highest value in sample No. 6 may be due to use of excessive phosphorous fertilizers. Application of phosphorus (P) is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of

the crop. Potassium fixation occurs when soils dry and the potassium is bonded between layers of clay. Under certain conditions, dependent on the soil texture, intensity of drying, and initial amount of exchangeable potassium. From the analyzed samples potassium ranges from 108 Kg/hectare to 840 Kg/hectare (Fig. b) indicating sufficient K in most of the sample except sample No 2, 4 and 9.

The Copper is an essential micronutrient for normal plant growth. The copper content of most plant is generally between 2 and 20 ppm in the plants. As copper is strongly bound to soils it is very immobile and hence the plant roots are frequently higher in copper concentration than other plant tissues. In the soils under study the concentrations of Cu range from 2.02 ppm to 26.8 ppm (Fig .b).Iron is essential for chlorophyll and protein formation, photosynthesis, electron transfer oxidation and reduction of nitrates and sulphates and other enzyme activities. Iron is one of the most common nutrients for plant growth and development because it exists in low-soluble form that is hardly available for plants. Table 1 and (Fig. b) shows the variation of the Iron content from 1.08 ppm to 17.6 ppm in the soils from the area. Manganese has oxidation influenced by both chemical and microbiological factors. Its activities has many enzyme reaction involved in the metabolism of organic acids P and N it is also involved in the photosynthesis and protein synthesis and also, manganese function along with Fe [12] in formation of chlorophyll. Table 1 shows the variation of the manganese content in the soils from the area from 2.34 ppm to 9.94 ppm. Zincdeficient plants are sensitive to pathogenic fungal root diseases [6]. Improvement of Zn nutritional status of plants reduces the exudation of such compounds from roots and increases resistance to fungal root diseases. The zinc concentrations range from 0.20 ppm to 1.54 ppm (Fig.b) indicating that in most of samples Zn is higher than the normal range of soils. The concentrations of boron in the soils range from 0.14 ppm to 2.05 ppm.(Fig. b).

3.2. Narsari Area from Shrirampur Tehsil District Ahmednagar:

An examination of soil samples (Table 2) shows that the values for pH range from 7.16 to 8.01 (Fig.c) indicating that the soils are alkaline and under such conditions the solubility of minerals decreases creating nutrient deficiencies in the soils. Plant growth is therefore limited by deficiencies in iron, manganese, zinc, copper and boron. Electrical Conductivity value ranges from 0.11 mS/cm to 0.50 mS/cm (Table 1), however sample No. 1 shows excess content of soluble salts which may due to excess use of fertilizer like K. Electrical conductivity is used to estimate the soluble salt concentrations in soil and is commonly used as a measure of salinity. Soil with EC below 0.4mS/cm is considered marginally or non-saline, while soils above 0.8 mS/cm are considered severely saline. The soils under analysis were found moderately saline except sample Nos. 4, 6, and 5 (Fig. c).

The organic carbon (%) ranges from 0.34 to 1.53 % (Fig.c).The organic soil matter includes all the dead plant materials and live or dead animals. Most living things in soils, including plants, insects, bacteria and fungi, are dependent on organic matter for nutrients and energy. Soils have varying organic compounds in varying degrees of decomposition. Organic matter holds soils open, allowing the infiltration of air and water, and may hold as much as twice its weight in water. Phosphorus is one of the key macronutrient required for plant growth and metabolism. Inorganic phosphate supplied to the soil as a fertilizer is rapidly converted into unavailable form. Soluble P converted into insoluble phosphate involves microorganisms. Phosphorous in the present soils vary from 10.23 Kg/hectare to 86.30 Kg/hectare (Fig. c) the highest value in sample No. 9 may be due to use of excessive phosphorous fertilizers. Application of phosphorus (P) is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crop. Potassium fixation occurs when soils dry and the potassium is bonded between layers of clay. Under certain conditions, dependent on the soil texture, intensity of drying, and initial amount of exchangeable potassium. From the analyzed samples potassium ranges from 30.91 Kg/hectare to 221.1 Kg/hectare (Fig. b) indicating sufficient K in most of the sample except sample No 1, 2 and 3.

The Copper is an essential micronutrient for normal plant growth. The copper content of most plant is generally between 2 and 20 ppm in the plants. As copper is strongly bound to soils it is very immobile and hence the plant roots are frequently higher in copper concentration than other plant tissues. In the soils under study the concentrations of Cu range from 0.29 ppm to 2.59 ppm (Fig .c).Iron is essential for chlorophyll and protein formation, photosynthesis, electron transfer oxidation and reduction of nitrates and sulphates and other enzyme activities. Iron is one of the most common nutrients for plant growth and development because it exists in low-soluble form that is hardly available for plants. Table 2 and Figure c shows the variation of the Iron content from 0.18 ppm to 2.77 ppm in the soils from the area. Manganese has oxidation influenced by both chemical and microbiological factors. Its activities has many enzyme reaction involved in the metabolism of organic acids P and N it is also involved in the photosynthesis and

protein synthesis and also, manganese function along with Fe [12] in formation of chlorophyll. Table 2 shows the variation of the manganese content in the soils from the area from 0.34 ppm to 6.11 ppm. Zinc deficient plants are sensitive to pathogenic fungal root diseases [6]. Improvement of Zn nutritional status of plants reduces the exudation of such compounds from roots and increases resistance to fungal root diseases. The zinc concentrations range from 0.26 ppm to 1.08 ppm (Fig. c) indicating that in most of samples Zn is higher than the normal range of soils. The concentrations of boron in the soils range from 0.08 ppm to 0.54 ppm.(Fig.c).

Table: 1: Showing the variation in different parameter of soil samples from Belapur of Shrirampur city

Sample No.	pH	E.C.	C-org	P	K	Cu	Fe	Mn	Zn	B
1	8.42	0.55	0.97	126.4	392	6.62	5.62	2.84	0.49	0.29
2	8.11	0.66	1.09	101.1	280	25.24	1.08	7.50	0.20	0.14
3	7.55	0.76	1.50	126.8	528	16.51	8.16	2.66	0.57	0.62
4	8.40	0.21	0.79	231.4	168	2.20	6.86	6.00	0.78	2.05
5	8.30	0.66	1.21	149.6	392	26.8	5.48	9.94	0.48	0.80
6	7.78	3.02	1.06	172.9	840	9.10	17.6	3.28	0.59	0.56
7	7.32	0.23	0.97	145.0	448	13.8	3.04	2.34	1.54	0.66
8	8.24	0.38	0.85	319.0	448	4.06	9.94	9.80	0.64	1.08
9	8.44	0.66	1.00	189.7	224	2.02	3.08	2.36	0.94	0.74
10	8.52	0.20	0.38	340.0	108	2.02	5.24	3.88	0.32	0.99

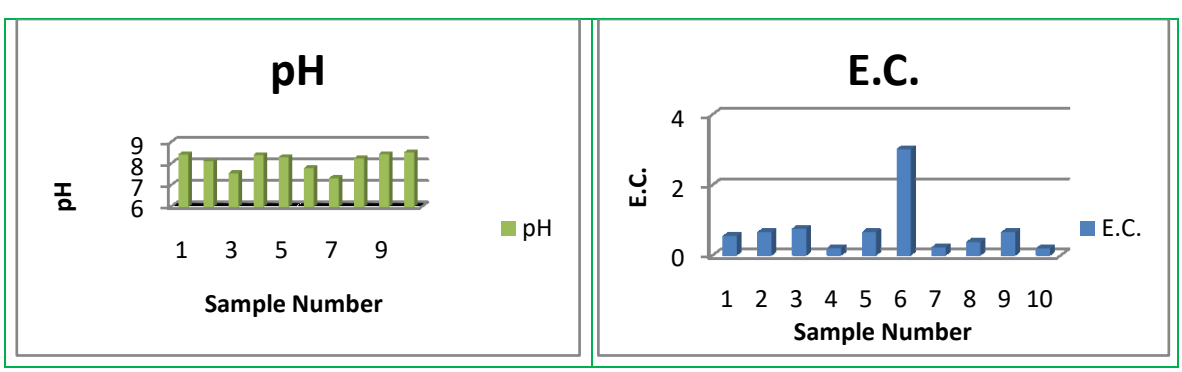
EC- mS/cm. C- org-%, P & K- Kg/hectre, Cu, Fe, Mn, Zn and B. - ppm

Table: 2: Showing the variation in different parameter of soil samples from Narsari of Shrirampur city

Sample No .	pH	E.C.	C-org	P	K	Cu	Fe	Mn	Zn	B
1	7.19	0.27	0.96	13.96	221.1	1.82	0.18	2.12	0.26	0.32
2	7.88	0.33	0.34	21.94	129.6	0.29	2.39	0.34	0.91	0.20
3	7.95	0.12	0.53	19.50	120.9	0.74	0.80	4.46	0.53	0.52
4	8.01	0.41	0.73	33.60	72.28	0.38	1.56	2.91	1.08	0.40
5	7.94	0.11	0.20	14.62	90.36	1.53	2.77	5.09	0.78	0.32
6	7.54	0.44	1.17	11.70	47.54	0.65	1.54	1.99	0.51	0.28
7	7.83	0.31	0.61	69.26	49.72	1.43	0.75	1.87	0.55	0.54
8	7.16	0.29	0.51	10.23	118.2	1.86	0.99	3.53	0.48	0.16
9	7.43	0.50	0.63	86.30	30.91	2.59	0.95	6.11	0.55	0.48
10	7.93	0.26	1.53	64.31	99.08	1.89	0.27	2.49	0.53	0.08

EC- mS/cm. C- org-%, P & K- Kg/hectre, Cu, Fe, Mn, Zn and B. - ppm

Figure: b). Variations of parameters like pH, EC, C-org, P, K, Cu, Fe, Mn, Zn, and Boron in soil sample from Belapur area.



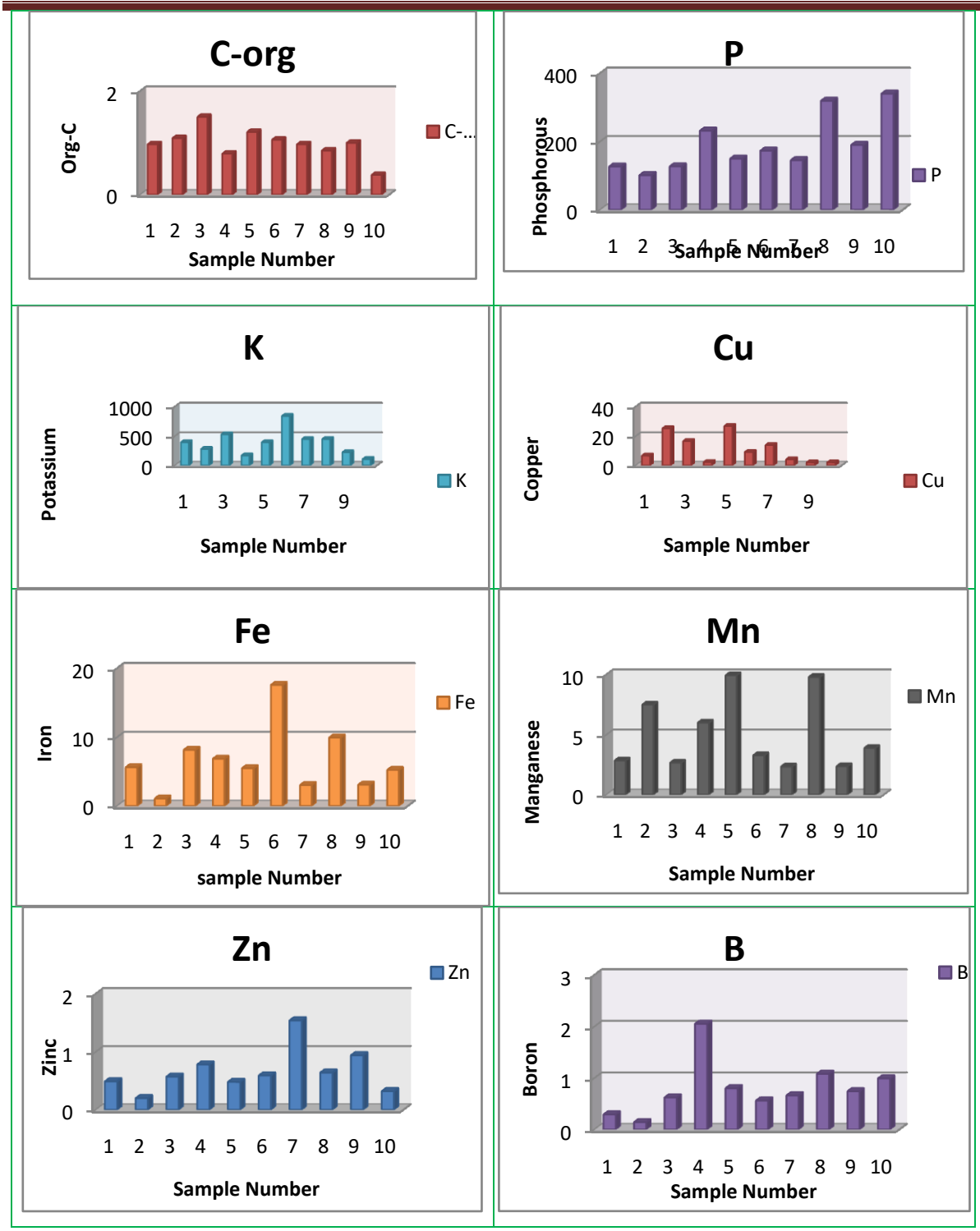


Figure: c) Variations of parameters like pH, EC, C-org, P, K, Cu, Fe, Mn, Zn, and Boron in soil samples from Nersari area.

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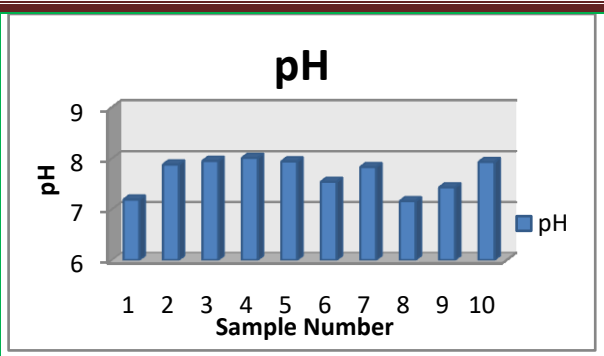

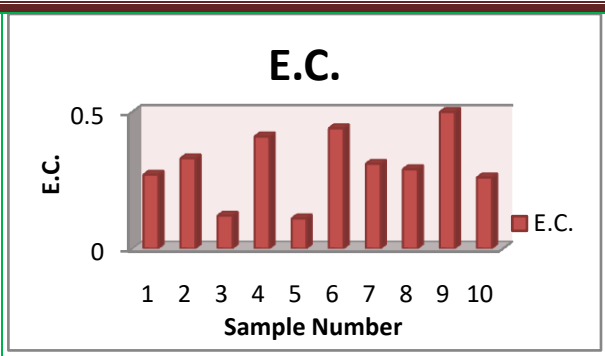

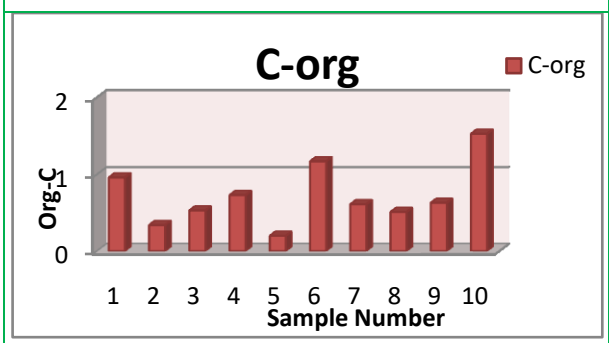

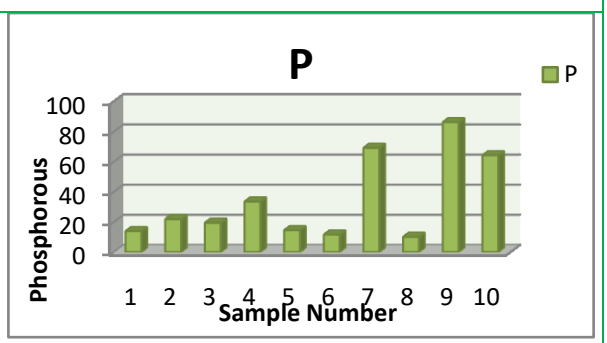

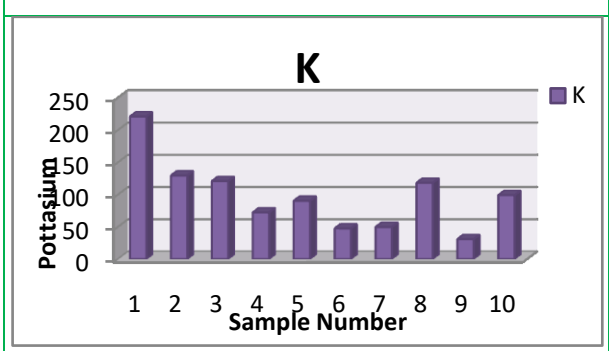

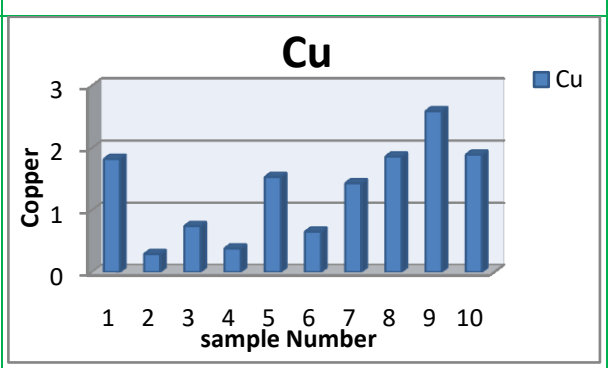

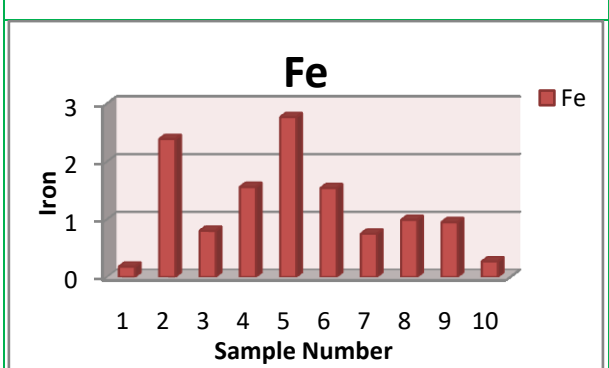

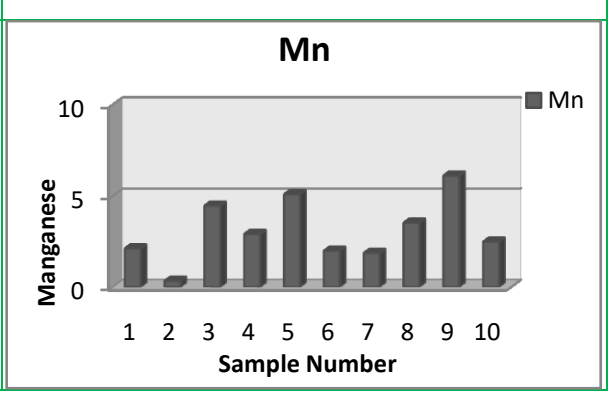

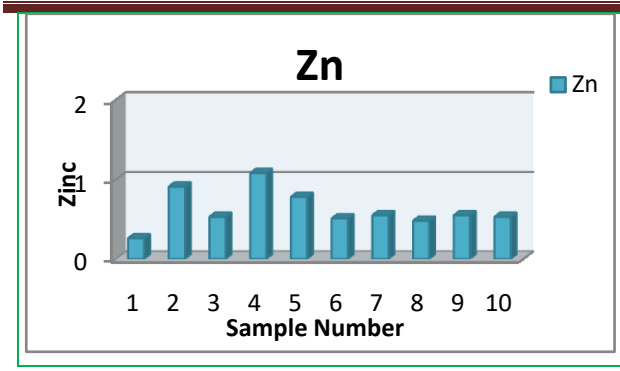

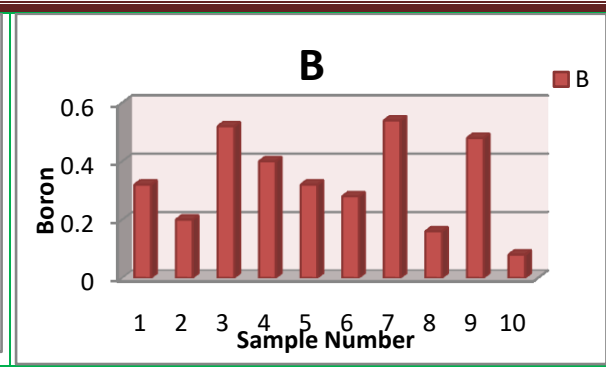

IV. Conclusions:

i. The Physico-chemical analysis of the soil samples from Belapur and Narsari villages towards Northern region of Shrirampur city have influence of the uncontrolled solid waste disposal practice as well as industrial effluents. The main crops are sugarcane and onion.

ii. Most of the farmers are using chemical fertilizers and the too much dose of such fertilizers in few soils has rendered high values of P and K. The retention of K could also be d clay minerals formed by chemical weathering of basalts which is the parent material for the soil.

iii. The values the Cu, Fe, Mn and Zn are Less than the normal range in most of samples of soil which could be due to poor drainage conditions in this area which also makes the soil alkaline

iv. Use of acidic fertilizers and organic manure can be a remedy which can raise the crop yield.

v. Monitoring of micronutrients in the soils should be done periodically as it can be an efficient way to assess the qualitative and quantitative abundances of the metal concentrations.

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$ZnCl_2$-GELA Catalyzed Multicomponent Synthesis of 1-(Benzothiazolylamino) methyl-2-Naphthols

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ABSTRACT: *One-pot, straightforward efficient multi-component condensation of aldehydes, 2-naphthol, and 2-aminobenzothiazole in the presence of ZnCl2-GEL as an effective acid catalyst for the synthesis of 1-(benzothiazolylamino)methyl-2-naphthol derivatives under thermal conditions is described. The present methodology offers noteworthy advantages such as mild condition, clean reaction profile, good yields, short reaction times, reusability of catalyst, and easy work-up.*

Keywords: *1-(Benzothiazolylamino)methyl-2-naphthols, ethanol, ZnCl2-GEL, multi-component reaction*

I. Introduction

Multi-component techniques have a wide application in the generation of series of complex organic molecules usually formed in the single step with diverse reaction substrates. Therefore, it is an attractive synthetic strategy [1-3]. These products are biologically active and found in various natural products [4]. To obtain required medicinal and biologically active product, starting materials used with the multi-component reaction which reduces reaction time and increases the yield of products in comparison with normal multistep methods [5-6].

2-Aminobenzothiazole derivatives are a vital class of synthetic chemistry and prepared by the variety of substrates such as naphthols, quinolinones [7-8], or with alkyl amines [9-10]. Benzothiazole is a vital heterocyclic component that has been extensively used as key building blocks for pharmaceutical agents. In accumulation, some compounds with the moiety 2-aminobenzothiazole are a known significant class of medicinal and pharmaceutical chemistry such as antitumor [11], antidiabetic [12], anticancer agent[13], antiepileptic agent [14]. They are found in natural products and pharmaceuticals which shows exceptional biological activities [15].

Some reports raised for the synthesis of 1-(benzothiazolylamino)methyl-2-naphthols using Sodium dodecyl sulfate (SDS) [16], citric acid [17], maltose [18], sodium hydrogen sulfate[19], oxalic acid [20], Fe_3O_4@SiO_2-$ZrCl_2$-MNPs [21], succinimidinium hydrogen sulfate ([H-Suc]HSO_4) [22], Sodium lauryl sulfate [23], and sodium chloride in water [24].However, many of the existing protocol are efficient but some of them suffer from certain drawbacks such as tedious preparation of catalyst, longer reaction time, low yield, high reaction temperature and use of expensive catalyst. Therefore, the main objective of the present research was to develop an efficient protocol for the synthesis of 1-(benzothiazolylamino)methyl-2-naphthols.

The procedure of Lewis acids in organic preparation; particularly in catalysis is one of most speedily emerging areas in synthetic organic compounds [26]. The several types of Lewis acid catalyzed reactions have been grown and most of them practiced in industries. These reactions must be accepted firmly under moisture-free circumstances [27-28]. A fascinating improvement in this respect is to exchange stoichiometric methods containing harmful reagents with cleaner substitutions [29]. In this regard, we projected that the imprisonment of Lewis acids in the medium of agar-agar, the concept acronymed as gel entrapped Lewis acids (GELA), can verify to be greatly striking scheme to improve the difficulties related with Lewis acids. The striking properties of $ZnCl_2$-GELA catalyst are air-stable, moisture insensitive, recyclable, heterogeneous, and biocompatible. In continuation of our research work [30], herein we report a green, one-pot, efficient method for the synthesis of 1-(benzothiazolylamino)methyl-2-naphthols catalyzed by using $ZnCl_2$-GELA in ethanol under reflux conditions.

II. Experimental:

All the chemicals were obtained from Sigma Aldrich, Spectrochem and Thomas Baker. These chemicals were used without further purification. In dried glassware, all the reactions were carried out. Agilent FT-IR was used for functional group analysis. Bruker AC NMR spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) was used for NMR spectra using $CDCl_3$ as solvent and chemical shifts (δ) are

mentioned in parts per million (ppm) values and TMS as the internal reference as well as coupling constants are expressed in hertz (Hz). Melting point apparatus with an open capillary was used for determination of melting point and were unchanged. Shimadzu QP2010 GCMS was used for recording mass spectra. Thin layer chromatography (TLC) was performed on silica gel polygram SIL G/UV 254 plates.

2.1 Preparation of Gel Entrapped ZnCl2 (ZnCl2-GELA):

In 100 mL round bottom flask, the boiling mixture of agar-agar powder (5 gm) in water (50 mL) taken and $ZnCl_2$ (2.5 gm) in water (5 mL) added in it. The resultant mixture was cooled in ice bath to obtain gel entrapped $ZnCl_2$ which acronymed as $ZnCl_2$-GELA**.**

2.2 General Procedure for the Preparation of 1-(benzothiazolyamino)methyl-2-naphthols:

A mixture of aromatic aldehydes (1 mmol), 2-naphthol (1 mmol), 2-aminobenzothiazole (1 mmol) and $ZnCl_2$-GELA (10 mol %) was refluxed in ethanol. After completion of the reaction confirmed by TLC, the product precipitated was separated by the filtration. The obtained product was washed with ethanol for two times to get it practically authentic. The crude product synthesized was purified by silica gel column chromatography (Merck, 60-120 mesh, ethyl acetate: petroleum ether as an eluent) to obtain the anticipated product, which was then characterized by different spectroscopic methods.

2.3Spectral data of novel compounds

4-[(benzo[d]thiazol-2-ylamino)(4-hydroxy-3,5-dimethoxyphenyl)methyl]naphthalen-2-ol (Table 3, entry 9) : M.P. 152-154 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 9.34 (brs, 1H, OH), 8.58 (s, 1H, OH), 8.06 (s, 1H, NH), 6.93-7.09 (m, 6H, Ar-H), 6.69-6.84 (m, 6H, Ar-H), 5.61 (s, 1H, Ar-CH), 3.63 (s, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.0, 158.9, 150.1, 148.5, 138.9, 137.3, 134.1, 132.7, 130.5, 130.4, 130.2, 128.6, 126.7, 126.6, 126.5, 125.5, 123.3, 118.7, 116.9, 108.5, 55.1, 54.5; IR (cm^{-1}): 3361, 3098, 2333, 1583, 1518, 1452, 1321, 1212, 1110, 990, 812, 738; MS (EI): *m/z* 458.52 $[M]^+$.

4,4'-[(dibenzo[d]thiazol-2,2'-ylamino)(phenyl)dimethyl]-dinaphthalen-2,2'-ol (Table 3, entry 11) :M.P. 234-236 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 9.22 (s, 2H, OH), 8.36-8.42 (m, 7H, Ar-H), 8.20-8.23 (m, 4H, Ar-H), 8.01-8.10 (m, 4H, Ar-H), 7.87-7.90 (m, 3H, Ar-H), 7.45-7.53 (m, 3H, Ar-H), 7.28-7.43 (m, 3H, Ar-H), 6.22 (s, 2H, Ar-CH), 5.48 (brs, 2H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.0, 158.1, 150.0, 142.5, 134.1, 132.5, 131.9, 130.5, 130.3, 129.8, 129.5, 128.0, 126.4, 126.1, 125.7, 125.6, 121.4, 119.1, 118.1, 117.1, 111.6, 55.7; IR (cm^{-1}): 3379, 3193, 2228, 1542, 1507, 1434, 1326, 1269, 1203, 816, 752; MS (EI): *m/z* 686.85 $[M]^+$.

III. Result and Discussion:

Our early efforts were concentrated on optimization of reaction circumstances and a new method of gel entrapped Lewis acid. $ZnCl_2$ was used as a Lewis acid in this scheme. Initially, the reaction of 4-chlorobenzaldehyde (1 mmol), 2-naphthol (1 mmol) and 2-aminobenzothiazole (1 mmol) taken as the model reaction for investigating various polar and non-polar solvents by the catalytic quantity of $ZnCl_2$-GELA (10 mol %). Solvent optimization clearly indicates that ethanol is the best solvent for this multicomponent reaction (Table 1, Entry 9). We found poor yield using non-polar solvents like 1, 4-Dioxane, toluene, chloroform (Table 1, Entry 1-3) and moderate yield with aprotic solvent like ethylene dichloride, THF, acetonitrile (Table 1, Entry 4-6). However, we observed better product yield using water and methanol (Table 1, Entry 7-8).

Table 1: Optimization of reaction condition using different solvents[a]

Entry	Solvent	Time (min)	Yield (%)[b]
1	1, 4-Dioxane	20	35
2	Toluene	30	38
3	Chloroform	25	45
4	Ethylene dichloride	30	50
5	THF	20	70
6	Acetonitrile	30	75
7	Water	25	55
8	Methanol	20	56
9	Ethanol	10	95

[a]***Reaction condition:*** 4-chlorobenzaldehyde (1 mmol), 2-naphthol (1 mmol), 2-aminobenzothiazole (1 mmol) and $ZnCl_2$-GELA (10 mol %) was refluxed

[b]Isolated yields after column chromatography

We turned our attention towards the optimization of catalyst concentration. The quantity of $ZnCl_2$-GELA for the preparation of 1-(4-chlorobenzothiazolylamino) methyl-2-naphthol in ethanol were examined (Table 2). The best result was obtained by carrying out the reaction using 10 mol% of $ZnCl_2$-GELA under reflux in ethanol (Table 2, Entry 2).

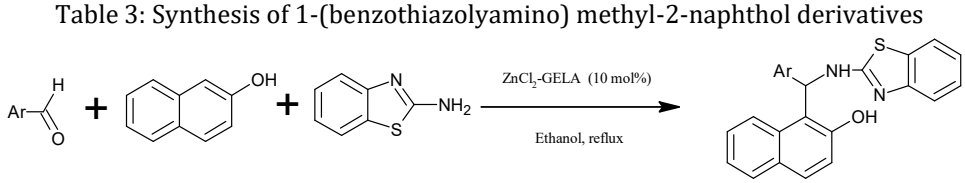
Table 2: Optimization of $ZnCl_2$-GELA concentration as the catalyst for the preparation of 1-(4-chlorobenzothiazolylamino) methyl-2-naphthol[a]

Entry	Concentration of catalyst (mol%)	Time (min)	Yield (%)[b]
1	5	10	82
2	10	10	95
3	15	10	95
4	20	10	95

[a]**Reaction condition:** 4-chlorobenzaldehyde (1 mmol), 2-naphthol (1 mmol), 2-aminobenzothiazole (1 mmol) and $ZnCl_2$-GELA under reflux in ethanol
[b]Yield refer to isolated pure products

Using optimized reaction condition, the generality of this reaction was studied with diverse aryl aldehydes as shown in Table 3, with both electron deficient and electron rich benzaldehyde (Table3, Entries 2-5 and 6), the anticipated products were obtained in good to excellent yields. The sterically hindered aldehyde such as 2,5-dimethoxybenzaldehyde, salicylaldehyde and syringaldehyde (Table 3, Entries 7-9) were also reacted with equal chemical efficiency giving desired product in excellent yield. Remarkably, heterocyclic thiophene aldehyde (Table 3, Entry 10) gives anticipated product in moderate yield. Furthermore, bifunctional aldehyde such as isophthaldehyde (Table 3, Entry 10) also reacted smoothly furnishing anticipated products in moderate yield.

Table 3: Synthesis of 1-(benzothiazolyamino) methyl-2-naphthol derivatives

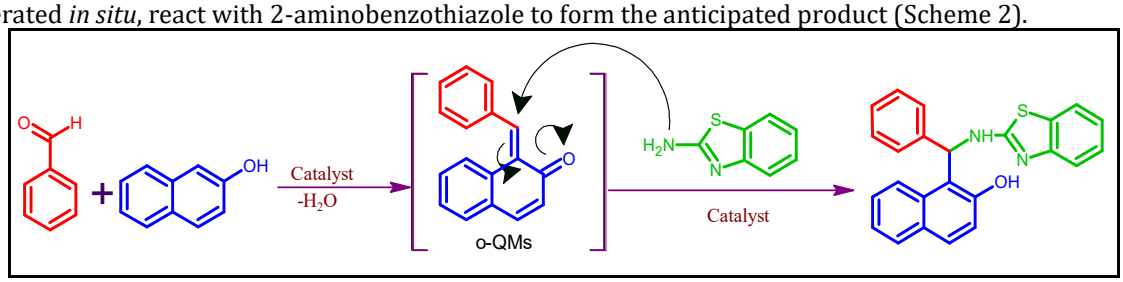


Ar= Aomatic group and heterocyclic ring

Entry	Aldehyde	Product	Time (min)	Yield (%)[a]	Observed MP (ºC)/ Literature MP (ºC)	Reference
1	Benzaldehyde	4a	9	90	202-204/ 202-203	[18]
2	4-Chlorobenzaldehyde	4b	10	95	208-210/ 209-210	[16]
3	4-Bromobenzaldehyde	4c	8	88	198-200/ 200-202	[20]
4	4-Nitrobenzaldehyde	4d	11	76	186-188/ 189-191	[17]
5	4-Cynobenzaldehyde	4e	10	88	213-215/ 215-217	[31]
6	4-Methoxybenzaldehyde	4f	8	90	174-176/ 175-176	[16]
7	2,5-Dimethoxybenzaldehyde	4g	10	92	208-210/ 209-211	[18]
8	2-Hydroxybenzaldehyde	4h	10	92	176-178/ 177-178	[18]
9	4-Hydroxy-3,5-dimethoxybenzaldehyde	4i	15	92	152-154	Novel
10	Thiophene-2-carbaldehyde	4j	12	92	190-192/ 191-193	[20]
11	Isophthaldehyed	4k	10	87	234-236	Novel

[a]Yields refer to isolated pure products.

As reported in the literature [21], the plausible mechanism of the reaction of 2-naphthol with aldehydes in the presence of $ZnCl_2$-GELA as the acid catalyst is known to give *o*-QMS. These *o*-QMS, generated *in situ*, react with 2-aminobenzothiazole to form the anticipated product (Scheme 2).



Scheme 2: Plausible mechanism for one pot synthesis of 1-(benzothiazolyamino) methyl-2-naphthols catalyzed by $ZnCl_2$-GELA.

The reusability and recyclability of the catalyst is a significant aspect from a green chemistry perspective and for industrial applications. To examine the probability of catalyst reusability, the template reaction using $ZnCl_2$-GELA in ethanol was carried out. As a result of this reaction, the catalyst recovered by filtration, washed with ethanol and recycled for another reaction with same reactants. The catalyst could be recycled for five runs without the obvious drop in the yield of the anticipated product.

To validate the merit of the existing work in comparison with the reported results, we compared the results of synthesis of 1-(benzothiazolylamino) methyl-2-naphthol derivatives as shown in Table 4. $ZnCl_2$-GELA (10 mol %) could act as an operational catalyst with respect to reaction time (Table 4). The advantages of $ZnCl_2$-GELA includes easy product separation, mild reaction circumstances and significantly less waste byproducts formation during reaction, shorter reaction period, reusability of catalyst. Therefore, the obtained results clearly revealed that $ZnCl_2$-GELA catalyzed reaction strategy could be better strategy compared with other catalytic reaction strategy.

Table 4: Comparison of the results of $ZnCl_2$-GELA with other catalysts used for synthesis of 1-(benzothiazolyamino) methyl-2-naphthol

Entry	Catalyst (Concentration)	Temperature (°C)	Time (min)	Yield[a] (%)
1	SDS (0.02 mmol)	100	60-300	71-93
2	$NaHSO_4.H_2O$ (0.01 mmol)	100	4-30	52-95
3	Maltose (0.02 mmol)	80	5-15	45-95
4	Citric acid (0.02 mmol)	80	5-15	52-92
5	Fumaric acid (0.02 mmol)	80	5-15	52-92
6	Oxalic acid (0.02 mmol)	80	5-30	57-95
7	$ZnCl_2$-GELA (0.01 mmol)	50	5-15	72-95

[a]Yields refer to isolated pure products

IV. Conclusion

In summary, a facile protocol was designed for the synthesis of 1-(benzothiazolylamino) methyl-2-naphthols under reflux condition. The most noticeable points for this methodology are mild reaction circumstances, great atom economy, shorter reaction times, higher product yield and reusability of catalyst. Therefore, this reaction approach make it valuable and striking features for the preparation of a wide variety of biologically active compounds.

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Use of ICT for Effective Teaching of Chemistry Concepts

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ABSTRACT: *Over the past few decades, the global economy has shifted from manufacturing–centric to a knowledge–driven. The contribution of hi-tech- manufacturing and high value–added services to the GDP has been increasing considerably. Success in leveraging knowledge and innovation is only possible with a sound infrastructure of higher education. A successful education policy forms the foundation of all fields of national development which include political, social, economic, technical, scientific, and environmental sector. Education in general and higher education in particular plays a vital role in the development of human potential and thereby national development. Recently, changes in science and technology have considerable impact on the higher education system which aims at developing the abilities of students to keep pace with changing world. Thus, higher the quality of university education in a country, more prosper and competitive are the people. The present method of teaching by conventional method using chalk and black board has intrinsic weakness. On the other hand, ICT in education has positive impact on students' conceptual understanding. By considering the advantages of multimedia computer programmes for effective teaching of chemistry concepts, researcher developed multimedia computer programme on organic chemistry concepts and used it for experimental group of first year science undergraduates. The multimedia programme was developed by using various media such as text, graphics, animation and sound. Teaching with use of multimedia programmes was beneficial to students of experimental group. It was confirmed by t-value. During this work, researcher administered pre-test, post-test, retention test, and sought opinion of students. Today, Government of India has been promoting inclusion of ICT in teaching-learning process. Therefore, programme developed by researcher will be very useful to such policy of Government. It will reduce cost of education considerably.*

I. Introduction

We are living in information age where knowledge and information are growing at a phenomenal rate. It is the rapid obsolescence of information. The skills and competencies that were good enough in yesteryears are no longer adequate today. The complexion of the current and emerging challenges calls for a new set of skills and dynamic optimistic approach for teaching-learning process. In India, there were just about 20 Universities and 500 Colleges at the time of independence. Today, this number has grown exponentially. There are 785 Universities, 38056 Colleges and 11922 Standalone Institutions. Quality teaching is the use of pedagogical techniques to produce learning outcome of students. It involves several dimensions, such as the effective design of curriculum and course content, a variety of learning context (including guided independent study, project-based learning, collaborative learning, experimentation, etc), soliciting and using feedback, and effective assessment of learning outcomes. It also enfolds well-adapted learning environments and student support services. Fostering quality teaching is a multilevel effort. Quality teaching takes place at three interdependent levels. At the institution level including projects such as policy design, support to organization and internal quality assurance systems. At the programme level it comprise actions to measure and enhance the design, content and delivery of the programmes within a department or a college. Support for quality teaching includes use of ICT in higher education especially for science undergraduate.

ICT can be divided into two components, Information and Communication Infrastructure (ICI) which refers to physical telecommunication system networks (cellular, broadcast, cable, satellite, postal) and the services (Inter Net, voice mail, radio and television). The Information Technology (IT) refers to the hardware and software of information collection, storage, processing and presentation. Teaching and Learning using ICT has become a reality in both the Conventional Education System and the Distance Education System. Literacy in ICT is essential to teach and to learn using ICT. Both the Teacher and the Lerner should be ICT literate. Educational ICT tools can be divided into three categories namely:

Input Source (Visualizer, Document Camera/PC, Slate/Tablet,Student Response System/Application Software),

Output Source (Projector/Interactive Whiteboard/Display, Monitor, TV),

Others (Digital Camera/Digital Recorder/Switcher/Other Technology).

"ICT manages large quantity of information and communicate the same to the concerned people. ICT is not limited to the computers or internet. It ranges from the use of FM radio to the use of satellite for

communication. It includes both the form and essence of communication"

Information and Communication Technology

The hardware, software, the methods and knowhow required or used in acquiring, storing, processing and displaying data and information is collectively known as the information technology (IT). Hardware, knowhow, programs and the methods are used to ensure that the message is transmitted correctly, efficiently and cost effectively (CT). Both of these technologies are designed for each other. Hence, IT and CT started moving together and new term was coined which is named as Information and Communication Technology (ICT).

According to UNESCO (2002) Information and Communication Technology (ICT) is defined as the combination of informatics technology with other related technologies, especially communication technology. It is defined as the technological applications (artefacts) of informatics in society. Informatics (Computing Science) is defined as the science dealing with the design, realization, evaluation, use and maintenance of information processing system. It includes hardware, software, organization and human aspects. It is industrial, commercial, governmental and political implications. It is also defined as a diverse set of technological tools used to create, disseminate, store and manage information and communicate. It includes computers, internet, broadcasting technology (radio and television) and telephony.

Considering interactive nature of multimedia computer for proper communication of organic chemistry concepts, researcher developed computer assisted learning package (multimedia software) and used it in teaching learning process for experimental group of B.Sc. Part-I students. Researcher developed this software by using various media such as text, graphics, animation and sound. The experimental group was benefited by this software. Researcher administered pre-test, post-test, retention test and elicited opinion of students. It has been observed that multimedia use in teaching –learning of organic chemistry enhanced the understanding of students at undergraduate level. The null hypothesis was tested on the basis of t value. Thus it is beneficial to use ICT for effective teaching-learning process.

II. Objectives of the Study

To develop multimedia computer assisted learning package on aromatic electrophilic substitution reaction for B. Sc. I students.

To compare the effectiveness of computer aided teaching-learning strategy with the traditional teaching method.

Limitations

In the present investigation, the students learning in B. Sc. Part-I in K. B. P. College during academic year 2017-18 were included.

The developed software program is based on the contents of point included in the syllabus of Shivaji University, Kolhapur (M.S.), India.

III. Null Hypothesis

H0.1 The experimental and control groups will not have different mean test scores.

Alternative Hypothesis

H1.1 The experimental and control groups will have different mean test scores.

Development of a product

The first step was to prepare layout and designing the contents to represent the same in interactive software program. Storey-boards were designed by the researcher. Text matter was developed in Microsoft Word which was imported in Macromedia Flash 5.0 where it was aligned frame by frame. Then frames and buttons were designed and developed. All the frames, buttons, pictures and still images were developed in Adobe Photoshop Cs and Adobe Illustrator 11.0. These were considered as a raw material for further designing. Then all the designed frames were imported into Macromedia Flash where all the contents were entered. The buttons and icons were given motion in the same software. The Macromedia Flash was utilized for motion animation and linkages of pages. The content frames were further published in the format of 'Application Files' (.exe). The verbal narration sound was recorded and edited in Sony Sound Forge 8.0.

IV. Design of the Study

In the present research study the investigator followed experimental research design. At the beginning, a diagnostic test was administered on 60 students learning in B. Sc. Part I in K. B. P. College, Urun Islampur,

District Sangli, M. S. India. A pre-test question paper was set for 50 marks containing 50 questions with multiple choices and each question was carrying 1 mark. All the questions were based on the chemistry syllabus at +2 level.

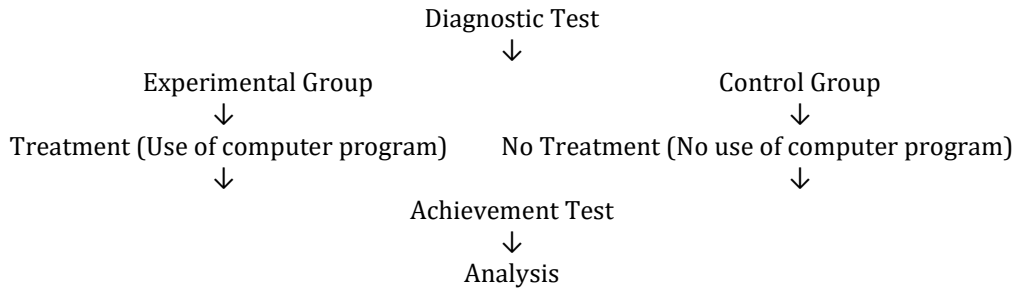

Figure1. Experimental research design

Table1. Comparison of performance of groups in achievement test

Group	Number of students	Mean test score	Variance	Standard deviation	t value
Experimental	30	23.9	13.74	3.7	2.69
Control	30	21.0	6.96	2.63	

The t test, the test of the significance of the difference between two means is tested at 0.01 level.

Based on the diagnostic test scores, the students were distributed into two equivalent groups. While forming two equivalent groups, the male and female students with equal score were distributed in both experimental and control groups. The experimental group was taught with the help of computer program. Thus they received treatment. The control group was taught with the traditional method of teaching. They did not receive treatment. At the end of the experiment, the achievement test was administered on both groups and their performance in achievement test was analyzed. The questions asked in the achievement test were objective questions with multiple choices, match the pair, questions for short answer and questions for long answers. The total marks allotted for the test were 50. The validity of both diagnostic and achievement tests was done properly.

The mean test score of the experimental group was 23.9 and that of control group 21.0. The mean test scores indicated that there is significant difference between achievement test scores of these groups. The test of the significance of the difference between two means t test has the value 2.69 which exceeds 1.96. Therefore, the null hypothesis is rejected at the 0.01 level of significance and alternative hypothesis H1 is accepted.

V. Discussion of Results

In the present investigation, researcher found that the mean test score of achievement test of experimental group was higher than the control group. The students of the experimental group acquired more knowledge of the concept than the students of the control group. Therefore, incorporation of multimedia program in teaching-learning process results into better performance of the students. It enhances the learning and understanding of concepts of organic chemistry such as aromatic electrophilic substitution reaction.

Thus it is concluded that, use of multimedia program in organic chemistry teaching-learning process is beneficial and may be used as a supporting tool to traditional classroom teaching method. It may be used as per the need and situation of the students.

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Efficient synthesis of 4H-chromene derivatives using Schiff base metal complex as catalyst

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ABSTRACT: *Rapid and efficient synthesis of 4H-chromene derivatives have been achieved by reaction of dimedone, malononitrile and aryl aldehydes catalyzed by Schiff base metal complex. Good yields, short reaction time, purity of products are the noteworthy aspects of the protocol. Schiff's base prepared is condensation product of salicylaldehyde and p-toluidine, various, metal complexes were prepared by reaction of Schiff base ligand and metal salts like $ZnCl_2$, $CoCl_2$, $NiCl_2$.*

Keywords: *Chromene, Schiff's base, catalyst*

I. Introduction

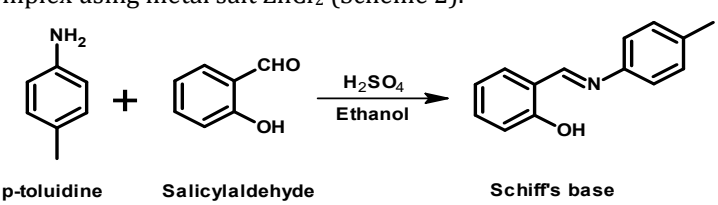
Metal complexes of Schiff's bases prepared from reaction of salicylaldehyde with primary amines have been extensively studied.[1-3] Chemistry of transition metal Schiff base chelates have accentuated research community for last few decades by displaying catalytic activity in reactions such as carbonylation, hydroformylation, reduction, oxidation, epoxidation and hydrolysis.[4-9] They also displayed significant antifungal and antibacterial activities. [10-11]

4H-chromene derivatives have enticed researchers due to their spasmolytic, diuretic, anti-coagulant, anti-cancer and anti-anaphylactic activities. [12-14] They also constitute several natural products. [15-16] 4H-chromene also displayed potential applications to the treatment of human neurodegenerative disorders, including Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, AIDS associated dementia and Down's syndrome.[17] Thus, their potential applications enthralled modern researchers, academicians towards developing new methods for their synthesis which follow the green chemistry principle.

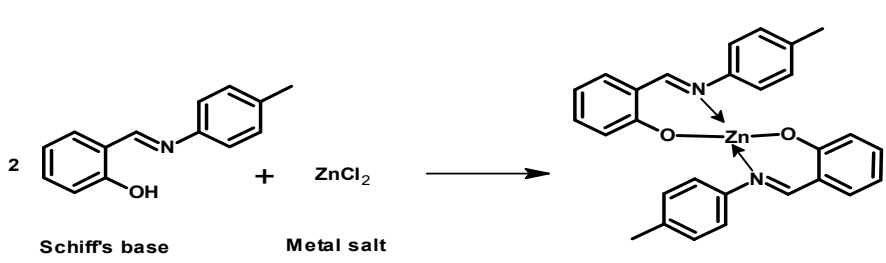
Hence, in this report we put forward a sincere attempt for synthesis of 4H-chromenes by reaction of dimedone, malononitrile and aryl aldehydes in presence of Schiff's base metal complex at room temperature.

II. Result and discussion:

Initially, we have synthesized Schiff's base by reacting p-toluidine and salicyaldehyde in presence of 2 drops of H_2SO_4 in ethanol(Scheme 1). Yellow colored product was obtained. Using this Schiff's base we prepared zinc metal complex using metal salt $ZnCl_2$ (Scheme 2).



Scheme 1: Synthesis of Schiff's base



Scheme 2: Synthesis of Metal complexes by reaction of Schiff's base ligand and metal salts.

IR spectra of Schiff's base ligand is compared with metal complex. Due to azomethine (C=N) group a characteristic band at 1625 cm^{-1} was observed. But in metal complex this band due to azomethine (C=N) group is shifted towards lower frequency 1615 cm^{-1} proves linkage of nitrogen to metal.

Band due to OH group of medium intensity around 3300-3400 cm^{-1} in Schiff's base was absent in metal complexes indicating linkage of phenolic oxygen to metal by deprotonation.

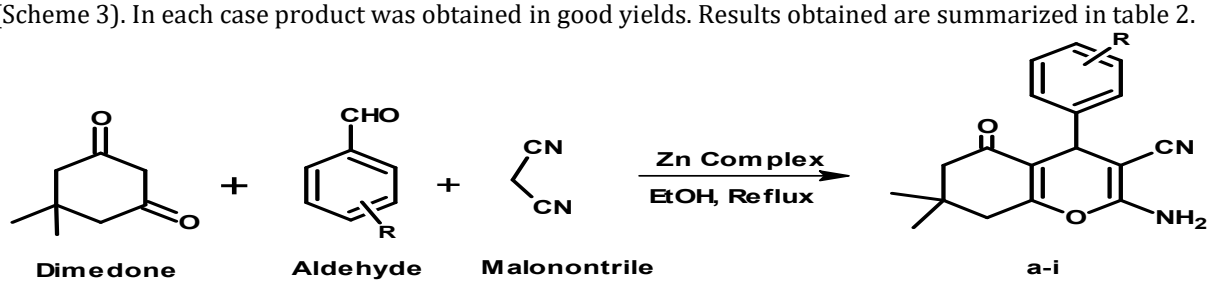
Furthermore, to prove the catalytic efficacy of these complexes, we demonstrated one pot multicomponent reaction of dimedone, malononitrile and benzaldehyde in presence of Zn complex in ethanol at room temperature. To our surprise reaction proceeded well and product was obtained in good yield after 2 hours (60%) (Table 1, entry 1). To increase the yield we carried out same reaction at reflux condition and found that yield increased to 85% within 30 min. (table 1, entry 2). To check the effect of solvent we carried out the same reaction in various combinations of water and ethanol but found ethanol as best solvent for reaction because in presence of water reaction mass becomes sticky (Table 1, entry 3-5).

Table 1: Synthesis of 4H-chromene derivatives at different conditions.[a]

Entry	Catalyst	Solvent	Temp.	Time	Yield[b]
1	Zn Metal complex	Ethanol	r.t.	2 hrs	60
2	Zn Metal complex	Ethanol	Reflux	30 min.	85
3	Zn Metal complex	Ethanol:Water (70:30)	Reflux	2 hrs	55
4	Zn Metal complex	Ethanol:Water (50:30)	Reflux	2 hrs	47
5	Zn Metal complex	Ethanol:Water (30:70)	Reflux	2 hrs	20

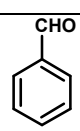
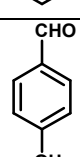
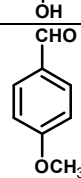
[a]The reaction was conducted at 1mmol of each substrate. [b]Isolated Yields.

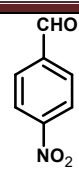
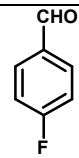
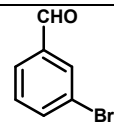
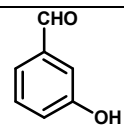
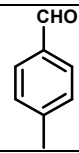
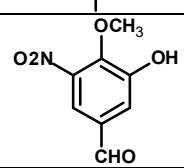
Thus, to check the versatility and generality of the protocol we reacted various substituted aryl aldehydes with dimedone and malononitrile in presence of Zn complex as catalyst and ethanol as solvent (Scheme 3). In each case product was obtained in good yields. Results obtained are summarized in table 2.



Scheme 3: Synthesis of 4H-chromene derivatives using Zinc Schiff's base complex as catalyst.

Table 2: Synthesis of 4H-chromene derivatives using Zinc Schiff's base complexas catalyst.[a]

Entry	Aryl aldehyde	Time	Yield[b]	M.P. (°C) obs/ M.P (C) lit. [References]
a		30	85	230/231-232 [18]
b		45	87	221-223/222-224 [18]
c		40	86	194-195/194-196 [18]

d		50	82	180-181/179-180 [18]
e		55	83	213/212-214 [18]
f		45	80	224-225/224-226 [18]
g		40	83	227-228/228-230 [18]
h		35	88	218-220/218-220 [18]
i		50	83	222-226 [Novel]

[a]The reaction was conducted at 1mmol of each substrate. [b]Isolated Yields.

III. Experimental:

3.1 General

All the chemicals used were obtained from Loba Chem. Progress of reaction was monitored by TLC (Silica gel 60 F_{254}). ^1H and ^{13}C spectra of isolated compounds were reported by using $CDCl_3$ and DMSO solvent on Brucker 300MHz spectrometer with TMS as an internal standard.. Melting points were recorded using Lab Star Apparatus.

3.2 General Procedure for the synthesis of Schiff's base.

Salicylaldehyde (20 mmole) and p-toluidine (20 mmole) was taken into round bottom flask. To the mixture 2 drops of conc. H_2SO_4 was added and refluxed the mixture for 20 min. yellow colored Schiff's base obtained was filtered and dried.

3.3 General Procedure for the synthesis of Schiff's base Zinc Metal complex.

4.30 gm (20 mmole) Schiff's base and 1.362 gm (10 mmole) of ZnCl2 salt was taken in round bottom flask and 20 ml of ethanol was added. The mixture was stirred well and added 2 ml of acetic acid to it. The mixture was refluxed for 2 hrs. Product obtained was filtered, dried and used as catalyst.

3.4 General Procedure for the synthesis of 4H-chromene derivatives.

In a round bottom flask dimedone (1 mmole), malononitrile (1 mmole) and aryl aldehyde (1 mmole) was taken. To the mixture 5 ml ethanol and Zinc metal complex catalyst was added and refluxed for respective time. The reaction was monitored by TLC. After completion the product was extracted from reaction mixture using ethyl acetate. Obtained product was proceeded for analysis.

3.5 Spectral data of representative compound:

Entry a: IR (KBr) max (cm^{-1}): 3744, 3334, 2188, 1651, 1364. ^1H NMR (DMSO, 300 MHz) δ (ppm): 1.07 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 2.19-2.42 (2H, m, CH_2), 3.93(2H,s,CH_2), 3.15 (2H, s, NH_2), 3.84 (3H, S, OCH_3), 4.32(1H, s, CH), 7.14-7.15(d, 2H, Ar-H), 7.26(1H, s, OH) . ^{13}C NMR (DMSO, 75 MHz): 27.35, 28.87, 32.23, 35.24, 50.52, 59.06, 69.71, 78.95, 79.39, 79.83, 113.49, 114.90, 116.34, 120.22, 128.07, 128.82, 137.52, 137.58, 157.56, 158.89, 162.52, 196.02.

Zn-Schiff's base Complex: IR (KBr) max (cm^{-1}): 1615 (characteristic C=N peak). ^1H NMR (DMSO, 300 MHz)

δ (ppm): 2.41 (s, 6H, 2-CH_3), 6.95-7.41 (m, 16H, Ar-H), 8.64(2H, s, CH).

IV. Conclusion:

Efficient synthesis of 4H-chromene derivatives using Zinc metal Schiff base metal complex as catalyst. Good Yields, easy procedure, efficient catalyst, mild reaction conditions are the noteworthy aspects of the protocol.

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APPLICATION OF QUALITY BY DESIGN (QbD) APPROACH IN DEGRADATION STUDY OF SIBUTRAMINE

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ABSTRACT: *In normal Forced degradation study the drug is subjected to extreme chemical and environmental conditions to determine product breakdown levels and preliminary degradation kinetics, and potential degradation products. As novelty in work we have applied QbD approach whereby critical quality attributes related to various process of degradation like hydrolytic, photolytic, thermal and oxidative are identified and optimized. Due to application of QbD approach for analytical method development along with validation additional accuracy and precision is seen in the final results.*

In present work a simple, precise, accurate and stability indicating UV-method have been developed and validated for estimation of Sibutramine. For analysis of Sibutramine absorbance maxima was found to be 239 nm. And Beer's range was found to 2-16µg/ml. For stability study conditions were optimized in such a way that degradation between 5-20% of Sibutramine can be achieved as per the ICH requirement.

Using QbD approach in degradation study for acid degradation 1N HCL, for alkali degradation 0.1 N sodium hydroxide, for oxidative degradation 0.1% hydrogen peroxide, for thermal degradation at 60°c temperature, for photolytic degradation long wavelength were used.

Keywords: *Sibutramine, Beer's law, Analytical method validation, Forced degradation, QbD*

I. Introduction

Forced degradation (FD) study is a process by which the natural degradation rate of a pharmaceutical product is artificially increased by the application additional stressful conditions. [1] FD studies help in identify reactions that may be responsible for causing degradation of pharmaceutical product. They are being used as part of the development strategy and an integral component of validating analytical methods that indicate stability and detect impurities which are formed during manufacture, storage, or use and their properties are different from the desired product with respect to activity, efficacy and safety. The analytical methods are developed for determine the degradation products formed during accelerated and long term stability studies. Degradent may be hazardous, as they may cause toxic effects like cytotoxicity, genotoxicity or carcinogenicity. Hence regulatory authorities are insisting on development of stability related analytical profile.

QbD is "a systematic approach to development that begins with predefined objectives and emphasizes understanding and control, based on sound science and quality risk management" The outcome of using QbD concepts is a well-understood product and process that consistently delivers its intended performance. In present work the QbD approach has been applied to analytical methods used in force degradation study.

QbD approach has been applied to analytical method like the UV method as well as methods used for force degradation for which conditions like hydrolytic, thermal, photolytic and oxidative are applied. [2] In all these methods identification of critical quality attributes, which can affect the end results are identified.

For example, in hydrolytic degradation study critical quality attributes will be strength of acid as well as alkali, its quantity and time for which exposure is done. If all these parameters are optimized as per analytical guidelines the generation of specific amount of degradation product is possible and along with validation of method would provide more amount of accuracy to the results of analytical method. [3]

II. MATERIAL AND METHOD

2.1 Instruments

A shimadzu UV-visible spectrophotometer (UV 1800 shimadzu) connected to computer loaded with spectra management software, 10 mm matched quartz cells were used for all the absorbance measurement.

2.2 Material

Sibutramine was obtained as gift sample from stride pharmaceuticals, Bangalore, India. All chemicals like HCL, methanol, sodium hydroxide etc. were of analytical grade.

2.3 Mobile phase

Methanol (100%) was used as mobile phase.

2.4 Stock solution

Standard stock solution of sibutramine (100µg/ml) was prepared and from this required concentration were used for analysis.

2.5 Determination of wavelength of maximum absorption

For selection of analytical wavelength, 8µg/ml solution of sibutramine was prepared by appropriate dilution of standard stock solution and scanned in the spectrum from 400 to 200 nm using diluent as blank. From the spectra of drug. λ_{max} of sibutramine, 239nm was selected for the analysis. (Fig. 1)

2.6 Preparation of working standard solutions

The Prepared stock solution was further diluted with methanol to get working standard solution

2.7 Preparation of calibration curve

It was observed that Beer's law is followed in concentration of 2 to 16µg/ml. (Table 1)

VALIDATION OF DEVELOPED METHOD

2.8 Linearity and range

For linearity study, different concentration solutions ranging from 2 to 16µg/ml were prepared by dilution of standard stock solution having concentration of different aliquots of stock solution, calibration plot is represented in (Fig. 2)

2.9 Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100%, and 120%) of the bulk sample of sibutramine.[3] (Table 2)

2.10 Precision

These precision of the proposed method was done by actual determination of three replicates of fixed concentration of the drug and finding out the absorbance by taking a fixed concentration of 8µg/ml by the proposed method. From these absorbance mean, Standard deviation, %R.S.D was calculated. Intra Day Assay The assay procedure was carried out in the same day in the duration of 2 hours to 3 hours at fixed concentration and the results were compared. Inter Day Assay procedure was carried out for single day with freshly prepared solution from stock solution at fixed concentration at 24hour interval. [3] (Table 3 and 4)

2.11 Robustness

This procedure was carried out by changing the analyst within the same laboratory and same instrument. Then results were compared. [3] (Table 5)

FORCE DEGRADATION STUDY

2.12 Acid degradation

First in a 100ml volumetric flask, accurately weighed 0.8mg of bulk drug was dissolved with methanol, as application of QbD approach the strength, volume and time were identified as critical quality attributes. It was observed that o.5 ml of 1N HCl and half hour time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines [4] (Table 6 and Fig. 3, 4)

2.13 Alkali degradation

First in a 100ml volumetric flask, accurately weighed 0.8mg of bulk drug was dissolved with methanol, as application of QbD approach the strength, volume and time were identified as critical quality attributes. It was observed that 0.5 ml of 0.1N sodium hydroxide and half hour time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines [2, 5] (Table 7 and Fig. 5, 6)

2.14 Photolytic degradation

First in a 2 different 100ml volumetric flask, accurately weighed 0.8mg of bulk drug was dissolved with few drops of methanol and volume was made by methanol in each volumetric flask respectively. Then first sample was kept at normal wavelength for half hour and second sample was kept at long wavelength for half an hour in UV cabinet. As application of QbD approach the range of wavelength, time were identified as critical quality attributes .and half hour time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines.[2,5] (Table 8 and Fig. 7, 8)

2.15 Thermal degradation

First in a 2 different 100ml volumetric flask, accurately weighed 0.8mg of bulk drug was dissolved with few drops of methanol and volume was made by methanol in each volumetric flask respectively. Then second sample was kept in hot air oven at 60˚C for 2 hour and first sample was kept at room temperature for 2 hours. As application of QbD approach the temperature, time were identified as critical quality attributes

and 2 hour time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines [2, 5] (Table 9 and Fig. 9, 10)

2.16 Oxidation with H_2O_2

In a 2 different 10ml volumetric flask, accurately weighed 1mg of bulk drug was dissolved with few drops of methanol and volume was made by methanol in each volumetric flask respectively. As application of QbD approach the strength, volume and time were identified as critical quality attributes. It was observed that 2 ml of 0.1% hydrogen peroxide and 12 minutes time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines [2, 5] (Table 10 and Fig. 11, 12)

III. RESULT AND DISCUSSION

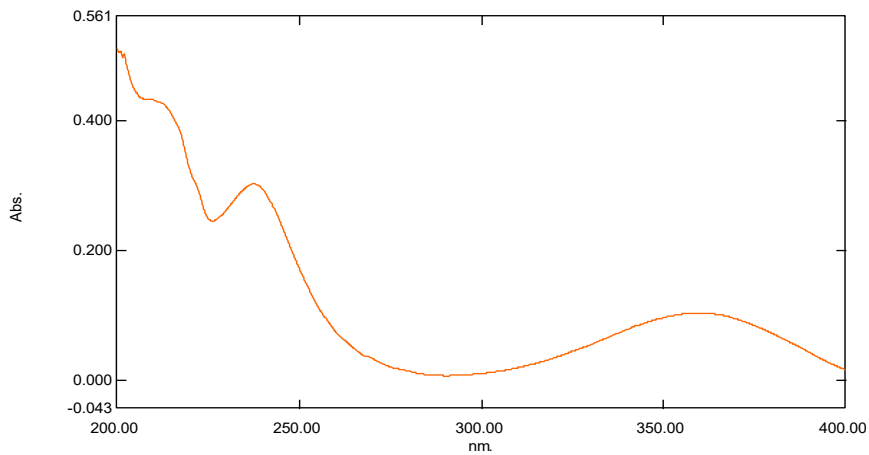

Fig 1: Typical UV Chromatogram Showing Sibutramine at 239nm

Table 1: Calibration range of sibutramine

CONCENTRATION (µ/ml)	ABSORBANCE (239 nm)
2	0.082
4	0.163
6	0.239
8	0.300
10	0.377
12	0.451
14	0.523
16	0.619

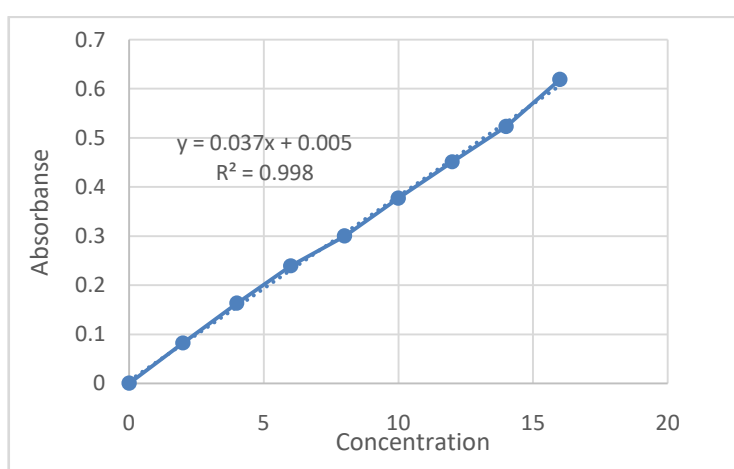

Fig 2: Linearity graph of sibutramine

Table 2: Accuracy reading of sibutramine

SR.NO	% DRUG SOLUTION	% DRUG CONCENTRATION			% RSD
1	80	67.5	68,12	68.75	0.009175
2	100	83.12	82.5	85	0.015583
3	120	102.5	101.25	101.5	0.006301

Table 3: Precision day 1 reading of sibutramine

SR.NO	TIME	% DRUG CONCENTRATION			% RSD
1	MORNING	81.2	80	77.5	0.029432
2	AFTERNOON	85	85.5	86.25	0.007351
3	EVENING	90	88.75	92.5	0.021118

Table 4: Precision day 2 reading of sibutramine

SR.NO	TIME	% DRUG CONCENTRATION			% RSD
1	MORNING	83.75	77.5	75	0.057231
2	AFTERNOON	88.75	86.25	88	0.014634
3	EVENING	101.25	103.75	102.25	0.012286

Table 5: Robustness reading of sibutramine

SR.NO	ANALYST	% DRUG CONCENTRATION			% RSD
1	1	87.5	86.25	86	0.009282
2	2	83.75	86.25	80	0.037749

Table 6: Acid Degradation study of sibutramine

SR.NO	CONC. µg/ml	NORMALITY (N) OF HCL	VOLUME OF HCL ADDED (ml)	% DEGRADATION
1	8	0.01	0.5	26
			1	46
			1.5	47
2		0.1	0.5	4
			1	21
			1.5	50
3		**1**	**0.5**	**20**
			1	35
			1.5	45

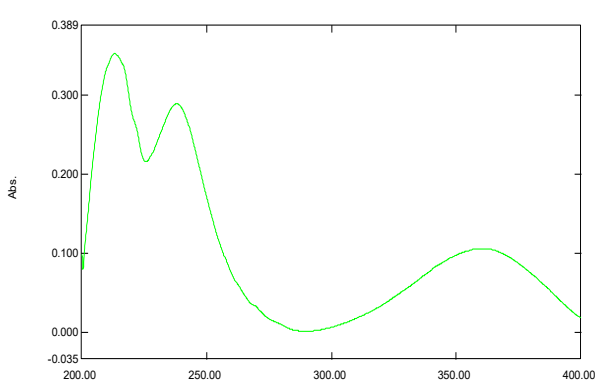

Fig 3: Spectra of sibutramine before acid degradation

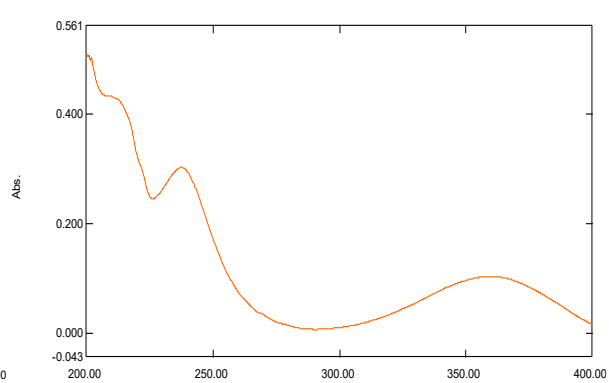

Fig 4: Spectra of sibutramine after acid degradation

Table 7: Alkali degradation study of sibutramine

SR.NO	CONC. µg/ml	NORMALITY (N) OF NaOH	VOLUME OF NaOH ADDED (ml)	% DEGRADATION
1	8	0.01	0.5	28
			1	46
			1.5	54
2		0.1	0.5	11
			1	31
			1.5	41
3		1	0.5	21
			1	42
			1.5	45

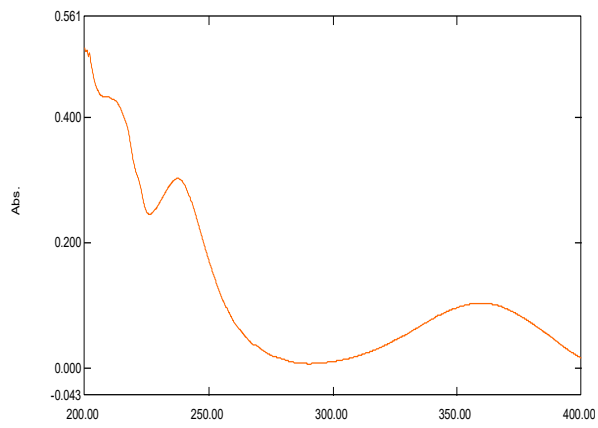

Fig 5: Spectra of sibutramine before alkali degradation

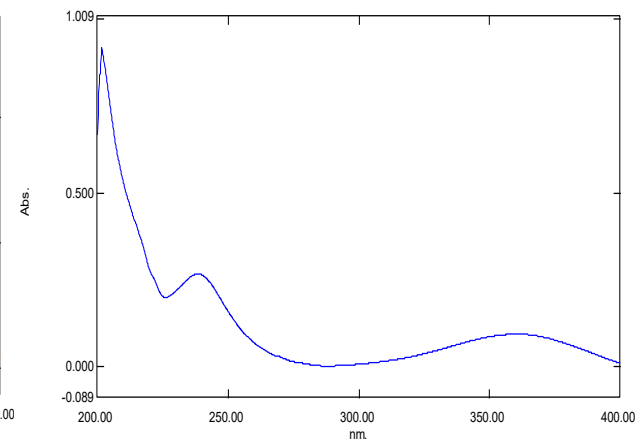

Fig 6: Spectra of sibutramine after alkali degradation

Table 8: Photolytic degradation of sibutramine

SR.NO	CONC. µg/ml	ABS. AT NORMAL WAVELENGTH	ABS. AT LONG WAVELENGTH	% DEGRADATION
1	8	0.173	0.162	7

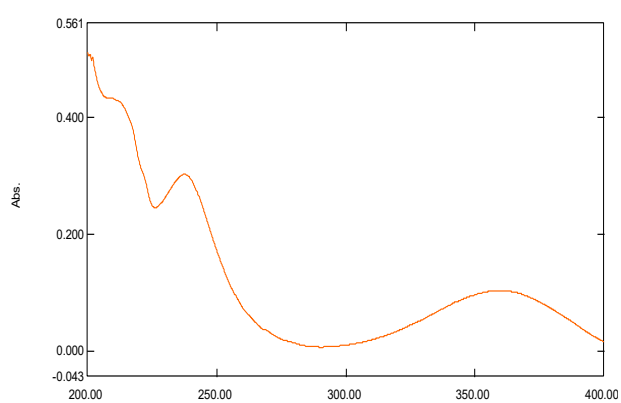

Fig 7: Spectra of sibutramine before photolytic degradation

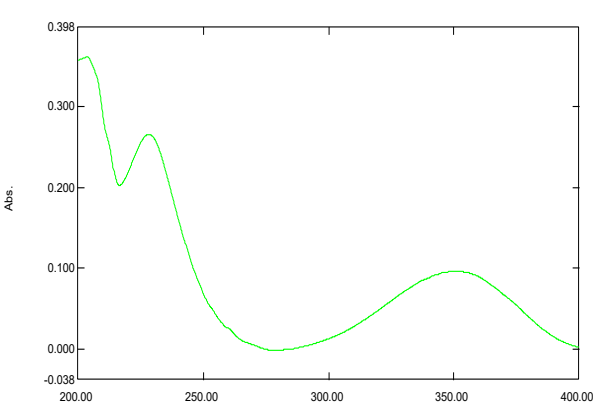

Fig 8: Spectra of sibutramine after photolytic degradation

Table 9: Thermal degradation of sibutramine

SR.NO	CONC. µg/ml	ABS. AT ROOM TEMPERATURE	ABS. AT TEMPERATURE (60°C)	% DEGRADATION
1	8	0.180	0.165	10

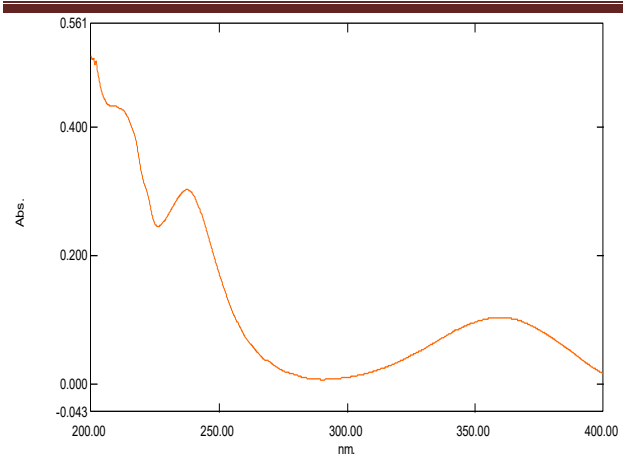

Fig 9: Spectra of sibutramine sample 1 photolytic degradation

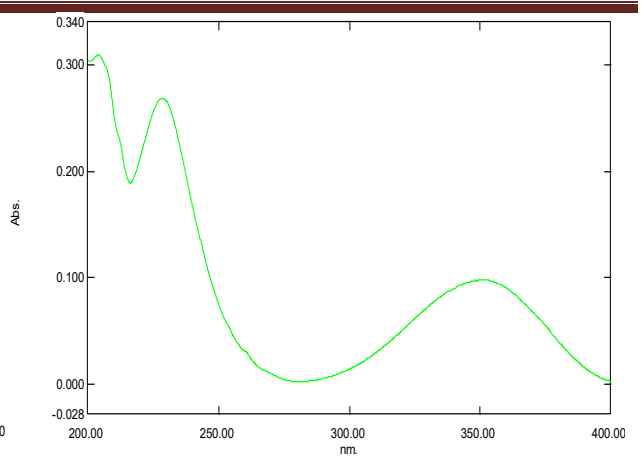

Fig 10: Spectra of sibutramine sample 2 photolytic degradation

Table 10: Oxidative degradation of sibutramine

SR.NO	CONC. µg/ml	% OF H_2O_2 ADDED	VOLUME OF H_2O_2 ADDED (ml)	% DEGRADATION
1	100	0.1	2	10
2	100	1	2	41

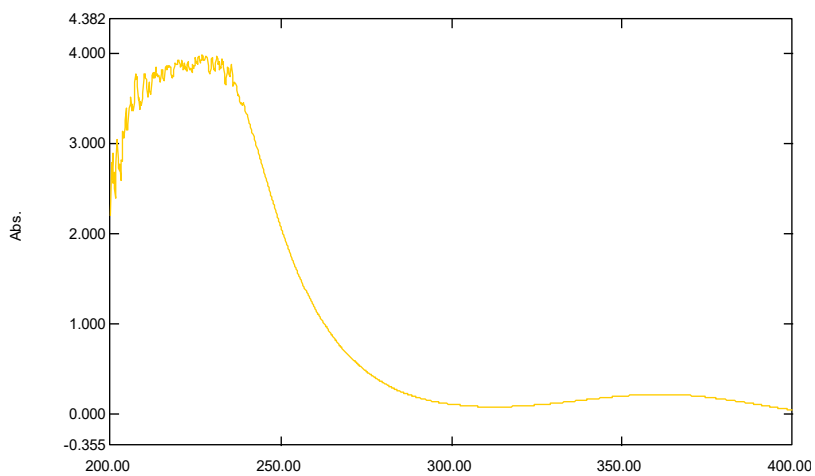

Fig 11: Spectra of sibutramine before oxidative degradation

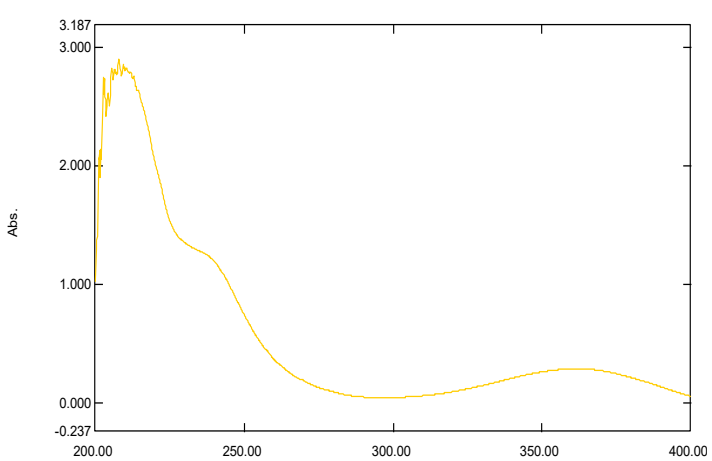

Fig 12: Spectra of sibutramine after oxidative degradation

IV. CONCLUSION

From this we conclude that the method developed and validated was linear, calibrated, accurate, precise, robust, and reproducible. From UV- spectroscopy we conclude that UV method was suitable for the degradation study.

From degradation study we conclude that sibutramine was degraded between 5-20% as per ICH guideline and it shown degradation at acid, alkali, photolytic, thermal and oxidative.

V. ACKNOWLEDGEMENT

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Catalytic Activities of Mixed Ligand Transition Metal Complexes Containing 1, 10-Phenanthroline, for N-Alkylation and N-Arylation of Heterocycles, Under Mild Conditions

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ABSTRACT: *The Schiff base mixed ligand transition metal complexes [M(L)(1,10-Phenanthroline)X_2] where L1 is PBADQ, L2 is 1, 10-Phenanthroline, M= Co, Ni, Cu, Zn, X= Cl and characterized by UV, IR, NMR, TGA-DSC, BET, ESR analysis and elemental analysis. These complexes were used as catalyst for N-Alkylation of a variety of heterocycles with 1, 3 dibromopropane and N-Arylation of different heterocycles with aryl halides (Ar-X, X=Cl, Br, I). All the complexes efficiently worked as catalysts.*

I. Introduction

In recent years, there has been renewed attention in the synthesis and study of mixed ligand transition metal complexes. The convenience aspects of these complexes have received their share of attention as these have found applications in diverse fields.[1] Chiral metal complexes are well known for their use as catalysts, especially in asymmetric synthesis.[2] Schiff base mixed ligand complexes differ from traditional complexes in the sense that they are having at least two different kinds of ligands associated with the same metal ion in a complex. The presence of more than one type of ligand in a complex increases chances of variation in properties expected for the complex. This makes the researcher interested in the synthesis of mixed ligand complexes with varying properties.[3] Synthesis and characterization of mixed ligand complexes is gaining importance day by day. The increased interest in this research area has motivated many researchers to get involved in this field. In recent years many publications are devoted to synthesis and characterization of mixed ligand complexes.[4-9] Mixed ligand complexes have been found to act as an active catalyst in reactions of industrial importance including hydrogenation, hydroformation, and oxidative hydrolysis of olefins and carboxylation of methanol. These complexes have also shown catalytic activity in various oxidation reactions of environmental and biological importance.[10-15] In the novel universal method N-alkylation of imidazoles and benzimidazoles occurs in the presence of alkaline reagents.[16]The reactions of imidazoles and benzimidazoles with an alkyl halide are commonly approved out in the presence of a hydroxide of an alkali melal or alkaline earlh element[17]sodium alkoxide or sodium amide with solvents such as ethanol, dioxane, toluene, xylene, or liquid ammonia. The N-alkylimidazoles and benzimidazoles can be prepared in a two phase system using crown ethers or quaternary ammonium salts as catalyst.[18] Recently Kikuyawas[19] described the N-alkylation of imidazoles and benzimidazoles by reaction with powdered potassium hydroxide in acetone using a slight excess of alkyl halide. N-Arylation of aromatic nitrogen in heterocyclic compounds is one of the most useful reaction types in medicinal chemistry[20] and natural products synthesis.[21] Theseheterocycles and their N-arylated products are the substructure of a variety of biologically[22] and pharmacologically[23] active substances. They have also found many applications in the chemistry of N-heterocyclic carbenes.[24]

II. Result and Discussion

The present effort reports synthesis and characterization of mixed ligand transition metal complexes with Schiff base PBADQ derived from 2-Aminobenzoyl hydrazide with benzaldehyde and 1,10phenanthroline as a co-ligand. Complex catalyst [Cu (L) (1,10-phenanthroline)]Cl_2 (fig.1) is thermally stable and it was investigated by Thermogravimetric Analysis (TGA) and Differential Scanning calorimeter (DSC) from the temperature range of 25-500 OC in an air atmosphere (Fig. 3). In the beginning, the slight weight loss of 0.71% observed in the temperature range of 25.00-90.750C was due to evaporation of physically adsorbed water molecules in the catalyst. The second weight loss in the temperature range of 202.70- 295.50OC (5.14 %) is attributed to decomposition of organic ligand. The chloride ions in the complex catalyst decompose in the thermal range of 295.50- 323.00OC which is reflected in the weight loss of 1.73 %. An abrupt weight loss

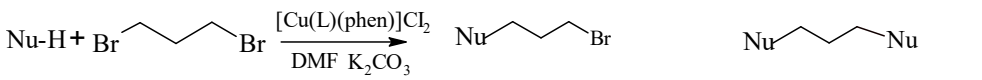
of 1.60 % in the thermal range 323.00-380.100C indicates the decomposition of second ligand 1, 10-phenanthroline. Finally the largest residual weight 84.16 % after thermal degradation of catalyst is in accordance with the oxides of copper. The catalytic activity of [Cu (L) (1,10-phenanthroline)]Cl_2, was compared with other synthesized Co (II), Ni(II), Zn(II) ions. It is noteworthy that the use of [Cu (L) (1,10-Phenanthroline)]Cl_2, has an excellent catalytic activity activity as compared to another complex catalytic systems.

The actual surface area can be calculated from acquaintance of the size and the number of the adsorbed gas molecules. Nitrogen is used most often to calculate BET surface, but if the surface area is very low, argon or krypton may be used as both present a more sensitive measurement, because of their lower saturation vapor pressures at liquid nitrogen temperature. Catalyst complex [Cu(L)(1,10-phenanthroline)Cl_2] was evaluated for determination of actual surface area during reaction . Surface area (asBET = 1.11 M2 g-1 total pore volume (P/Po=0.990) = 0.00479 cm^3g-1 and Mean pore diameter = 17.172 nm (fig. 4)

The magnetic moment of Cu (II) complex at room temperature lie down in the series of 2.55 B.M. This indicates that these complexes are monomeric in nature and the absence of metal–metal interaction. Applying this criterion the covalent character of the metal–ligand bond in the complexes under revise can be predicted. The trend g||>g⊥>g e (2.0023) experimental for these complexes shows that the unpaired electron is localized in dx2−y2 orbital of the Cu(II) ions and the spectral features are characteristics of axial symmetry[29]. The curve of the Cu (II) Schiff base mixed ligand complexes recorded.

To understand the correlation between electronic and geometrical properties of complex catalyst, its X band EPR spectrum was measured at 100 K in the solid-state (Fig. 5). The spectrum has been found to be axial and shows a well resolved hyperfine splitting of four bands for g|| corresponding to Cu (II) (I = 3/2) centre (g|| and g⊥values are 2.204 and 2.052, respectively). The fact that g|| > g⊥ > 2.0 and G = (g||− 2)/(g⊥− 2) = 3.9 with dx^2 -y^2 as ground state orbital

These mixed ligand transition metal complexes were screened for N-Alkylation of heterocycles with 1,3dibromopropane (scheme 1)

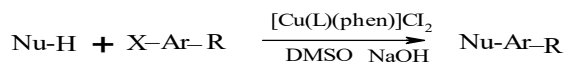


Coupling Ratio 1:1 (a)
Nu-H 1 Imidazole (1a)
2 Benzimidazole (2a)
3 Indole (3a)
4 Isatin (4a)

Coupling Ratio 2:1 (b)
Nu-H 1 Imidazole (1b)
2 Benzimidazole (2b)
3 Indole (3b)
4 Isatin (4b)

Scheme: 1 N-Alkylation of heterocycles

Catalytic activities of these four complexes in N-Alkylation of heterocycles with 1, 3 dibromopropane, complex [Cu (L) (1,10-phenanthroline)]Cl_2, K_2CO_3, DMF chosen for N-alkylation of heterocycles. Different heterocycles were treated with same coupling partner 1, 3 dibromopropane in 1:1 ratio then all gave better yield 73-92% (Table 1 entries 1-4). Especially indol and 1, 3 dibromopropane (1:1) gives towering product yield 92% (Table 1 entry 3). Simillarly the heterocycles such as imidazole, benzimidazole, indole and isatin moreover treated with similar coupling partner 1, 3 dibromopropane in 2:1 ratio which lead to product yield 57-69% (Table 1 entries 5-8). It was illustrious that [Cu (L) (1,10-phenanthroline)]Cl2 plays catalytic role in N-alkylated product yield elevated is 1:1 share of substrate than 2:1 ratio of substrate to reagent (1:1>2:1).The catalytic activity of Schiff base mixed ligand transition metal complexes 1-4 evaluated by using the C-N Coupling of aryl halides with heterocycles in the presence of NaOH(1.0 mmole) at 1000C in 1 mL DMSO (scheme 2).



5 6 7

Nu-H: Imidazole, Benzimidazole, Indole, Isatin
R= CH_3, NO_2 X= Cl, Br, I

Scheme 2 N- Arylation of Heterocycles

This catalytic system is used to study for N-Arylation of heterocycles with different aryl halides (Table 2). Imidazole treated with aryl halides (Ar-Cl, Ar-Br, Ar-I), Iodobenzene give better yield as compared to chlorobenzene and bromobenzene 90% (Table 2 entry 3). Finally iodobenzene, complex [Cu (L) (1,10-phenanthroline)]Cl2 NaOH, DMSO chosen for N-arylation of different heterocycles and substituted iodobenzene. Reactivity of substituted iodobenzene with electron donating and electron withdrawing group with imidazole, benzimidazole, indole and isatin using [Cu (L) (1,10-Phenanthroline)]Cl2 was in good health with electron withdrawing nitro group at p-position 4-nitro iodobenzene with better yield as compared electro donating methyl group at p- position 4- methyl iodobenzene (Table 2 entries 5,8,11,14). We have developed broad catalytic manner for N-alkylation and N-arylation of heterocycles such as imidazole, benzimidazole, indole and isatin with 1,3 dibromopropane and aryl halides Ar-Cl, Ar-Br, Ar-I respectively, promoted by Schiff base mixed ligand transition metal complex [Cu (L) (1,10-Phenanthroline)]Cl_2. The system is efficient for coupling N-alkylation heterocycles with 1, 3 dibromopropane to give moderate to excellent yield with share 1:1. (1:1>2:1). The introduction of one alkyl group in hetetocycles is much easier compared with double alkylation reactions. The system is efficient for coupling N-arylation of heterocycles with aryl halides modified with moderate to excellent yields.

III. EXPERIMENTAL

3.1 Synthesis of Schiff base

2-phenyl 3-benzoylamino, 1, 2 dihydroquinazolin-4(3H)-one. (PBADQ) synthesized from 2-Aminobenzoyl hydrazide by reported procedure [25]. The purity of the compound was checked by TLC on pre coated silica gel which gave a single spot to give Schiff base (PBADQ) yield 92% yellow powder.

IR (KBr) cm-1 υ1633(C=N), υ1706(C=O), υ 3336(N-H), 1H NMR (CDCl3, 300 MHz) δ9.25 (HC=N), δ 4.93(Ar-H), δ 3.64(N-H). UV ArC=C (π→π*) 250-260 λ max (nm), C=N (n→π*)315-320λmax (nm). Anal.Calcd for [C21H17N3O]: C, 77.04; H, 5.23; N, 12.84. Found: C, 77.00; H, 5.17; N, 12.79.

3.2Synthesis of Schiff base mixed ligand transition metal complexes

Mixed ligand transition metal complexes with 1, 10-Phenanthroline were synthesized by reported procedure[26].

Anal.Calcd for [Ni(L)(1,10-phen)Cl2] Yield 84%, C33H25N5OCl2Ni: C, 62.05; H, 3.88; N, 10.87; Ni, 9.21; Found: C, 62.20; H, 3.96; N, 10.98; Ni, 9.35; IR KBr cm-1 υ 1640 (C=O), υ 1616 (C=N), υ 3270 (N-H). NMR (CDCl3, 300 MHz) δ9.05 (HC=N), δ 7.98(N-H). UV Ar C=C (π→π*) 250-255 λ max (nm), C=N (n→π*) 300-320λmax (nm). (π→π*) or metal to ligand charge transfer (MLCT) transition 345-361 λmax (nm).

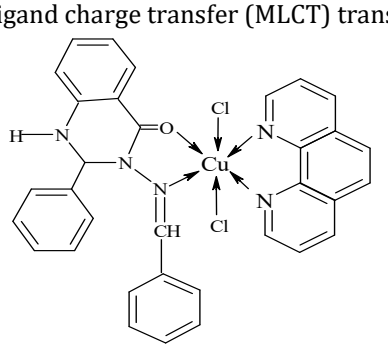


Fig. 1 Complex [Cu(L)(1, 10-phenanthroline)]Cl_2

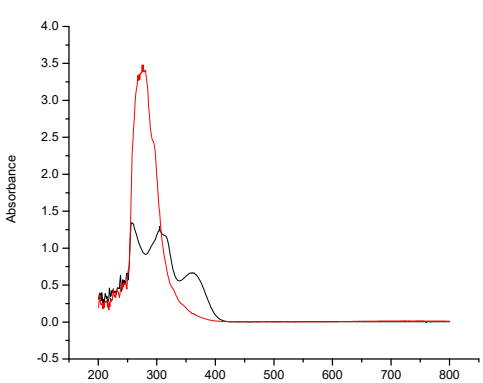


Fig.2 UV Spectrum of complex

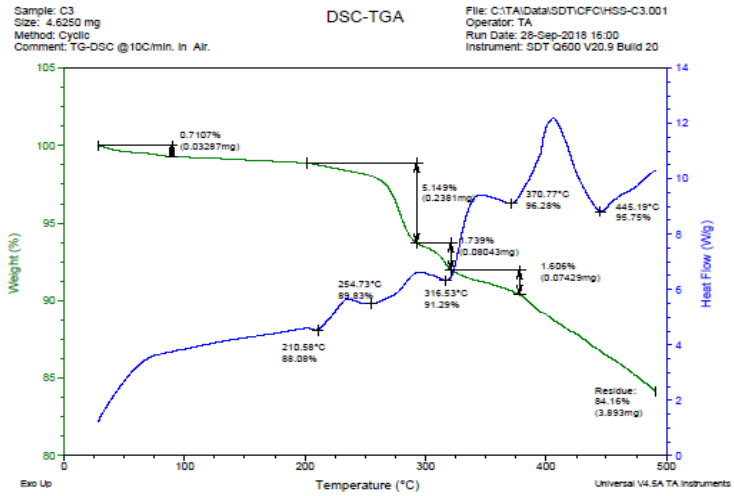


Fig.3 TGA-DSC curve of complex

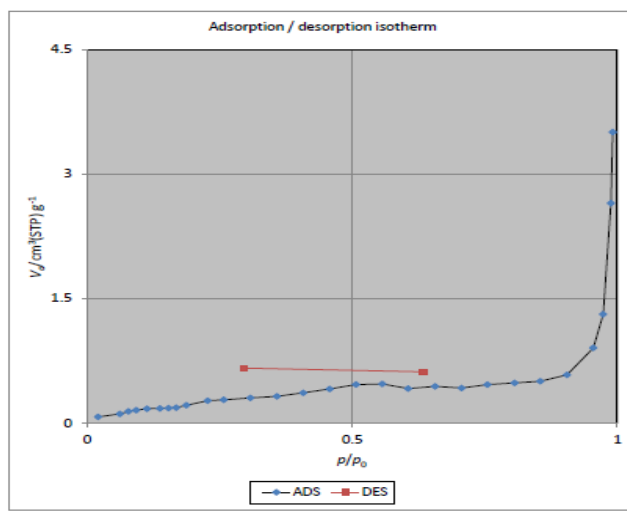


Fig. 4 Adsorption desorption isotherm of complex

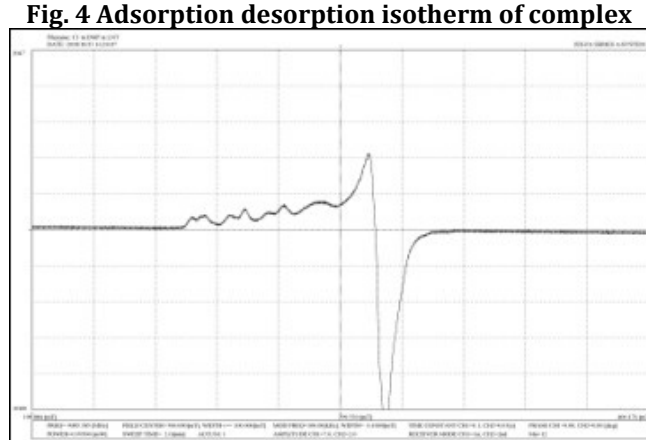


Fig 5. ESR Spectrum of complex

3.3 General procedure for N- Alkylation of Heterocycles with 1, 3 dibromopropane

According to a published procedure[27] N-alkyl product was prepared. Generally, the introduction of one alkyl group is much easier compared with double alkylation. N-(3-bromopropyl) indole (3a) -Indole and 1,3dibromopropane (1:1)

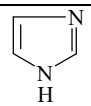
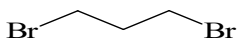
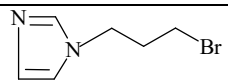
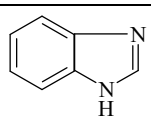
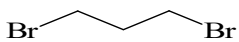
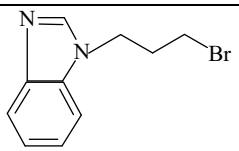
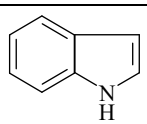
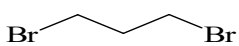
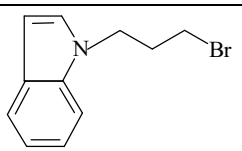
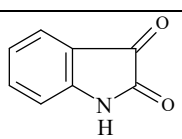
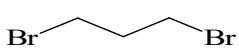
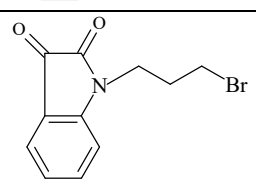
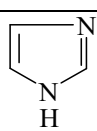
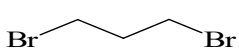
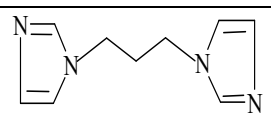
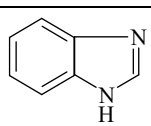
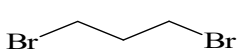
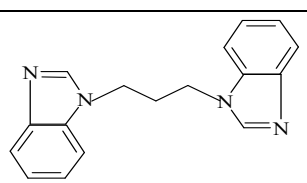
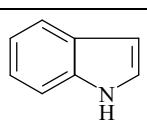
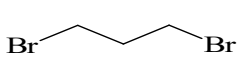
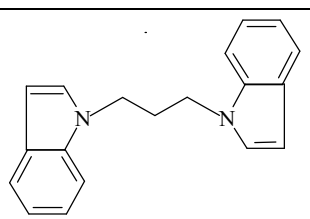
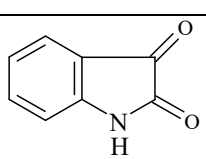
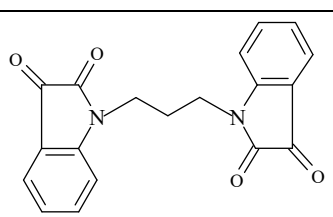
92% [$C_{11}H_{12}BrN$] GCMS (ES): m/z= 238[M] +. N-Propyl bis-indole (3b)-Indole and 1,3dibromopropane (2:1)

69% [$C_{19}H_{18}N_2$] GCMS (ES): m/z= 273 [M] +.

3.4 General procedure for N- Arylation of Heterocycles with Iodobenzene and its derivatives

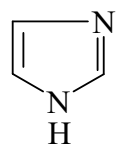
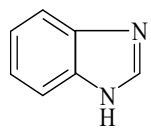
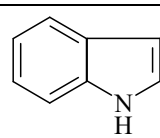
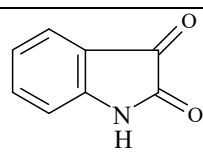
According to published procedure[28]. In round bottom flask, complexes [Cu(L)(1,10-Phenanthroline)Cl_2] (0.05 mmol), iodobenzene (0.5 mmol), imidazole (1.0mmol), NaOH (1.0mmol) and 1 ml DMSO was refluxed at 1000C in a preheated oil bath for 10 hrs. The reaction is monitored with TLC. N-Phenylindole (7i) 90% . GC-MS (EI): m/z = 193 [M] +

TABLE 1 N-ALKYLATION OF DIFFERENT HETEROCYCLES WITH 1, 3-DIBROMOPROPANE

Entry	HET-NH	COUPLING PARTNER	TARGET PRODUCT	YIELD %
1				73
2				77
3				92
4				80
5				57
6				62
7				69
8				68

Reaction conditions: coupling N-heterocycles with 1,3-dibromopropane (1:1) entries 1-4, and coupling as (2:1) entries 5-8.

Table 2 N-Arylation of different heterocycles with different substituted aryl halides

Entry	HeT-NH	R-Ar-X	Product	Yield %
1		Ar-Cl	7a	72
2		Ar-Br	7b	70
3		Ar-I	7c	**87**
4		4-CH_3,Ar-I	7d	52
5		4-NO_2,Ar-I	7e	58
6		Ar-I	7f	**82**
7		4-CH_3, Ar-I	7g	79
8		4-NO_2, Ar-I	7h	80
9		Ar-I	7i	**90**
10		4-CH_3, Ar-I	7j	60
11		4-NO_2, Ar-I	7k	67
12		Ar-I	7l	**74**
13		4-CH_3, Ar-I	7m	48
14		4-NO_2, Ar-I	7n	52

Reaction condition: coupling N-heterocycles (1 mmole), aryl halides (0.5 mmole), complex (0.05mmole), NaOH(1.0 mmole), DMSO (1 ml), 10 hrs at 100°C.

IV. ACKNOWLEDGEMENT

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Oxidation reaction catalyzed by Anderson type polyoxometalate catalyst

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ABSTRACT: *Polyoxometalates are having wide applicability in field of catalysis. Disulfides are biologically important molecules which can be easily synthesized by oxidation of thiols. In present work synthesis of disulfides were carried out by oxidizing thiols using Anderson type hexamolybdochromate(III) as catalyst. 30% H_2O_2 was used as an oxidant. The reaction was carried out at 60^0C in 50% aqueous acetonitrile solution. The reaction completed in short reaction time and gave high yield of disulfides.*

Keywords: *Oxidation, polyoxometalates, Anderson type, hydrogen peroxide*

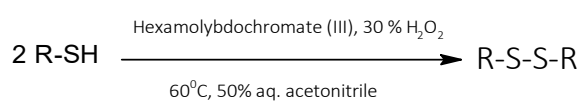
I. Introduction:

Disulfides are important molecules in field of biology [1]and synthetic organic chemistry [2]. Disulfides is used as a protecting group under oxidative condition for thiol and can be regenerated by S-S bond cleavage [3].Disulfides have many industrial applications and are important synthetic intermediates in organic synthesis [4].They are also useful as vulcanizing agents [5]. These molecules have potential applications in optical data processing and communication. Compounds containing a disulfide linkage have been used for the preparation of dynamic combinatorial libraries, macrocycles, dendrimers, rotaxanes and micelles. The wide applications of disulfides show that the synthesis of disulfide bonds is an important transformation in modern research [6].

Overoxidation of thiols results in formation of sulfoxides and sulfones. Therefore, controlled and selective methods have been developed for their oxidation. The most common and widely accepted method for preparation of disulfide is the oxidation of thiol. Hence several reagents [7] have been employed for this transformation.

Several features of heteropolyoxometalates make them economically and environmentally attractive catalysts. They have been effectively used in various organic transformation reactions. Most of the polyoxometalate catalysis reported was based on the utilization of Keggin or Dawson-type polyoxometalates and less attention is given to Anderson type structure.

In the present study, I report the successful utilization of Anderson type hexamolybdochromate (III) as an effective catalyst for oxidation of thiols to disulfides. The reaction was carried out in aqueous acetonitrile solution at 60^0C using hydrogen peroxide as oxidant, as shown in scheme 1.



II. Experimental

All the products are known compounds and were identified by comparison of their physical and spectral data with those of authentic samples. Thiols and other chemicals were purchased from Lancaster and BDH and used without further purification. Products were characterized by comparison of their physical data with those of known samples. Melting and boiling points were determined in an open capillary on electrothermal apparatus and are uncorrected. All yields refer to isolated yields.

2.1 Catalyst preparation

The catalyst sodium hexamolybdochromate(III)was prepared by the previously reported method [8]. 14.5 g of $Na_2MoO_4.2H_2O$ was dissolved in 30 ml of water and its pH was adjusted to 4.5 by using concentrated HNO_3. In another beaker 4.0 g of $Cr(NO_3)_3.9H_2O$ was dissolved in 5 ml of water. The solutions were mixed together and boiled for 1 min. Further it was filtered while hot. The filtrate was set aside for crystallization. The crystals started to appear in 1 hr. The solution was allowed to stand for 2 weeks. The precipitate was filtered off and washed several times with cold water. Reddish purple crystals were obtained.

2.2 Catalyst characterization

2.2.1 AAS analysis:

The complex $Na_3[CrMo_6O_{24}H_6].8H_2O$was studied by AAS analysis. 100 mg of catalyst sample was dissolved in 100 ml doubly glass-distilled water. 5 ml of this stock solution was diluted to 100 ml and used for AAS analysis of Cr and Mo metals using Perkin-Elmer AAnalyst-300. The complex $Na_3[CrMo_6O_{24}H_6]\bullet 8H_2O$shows (Theoretical): Na- 5.6303 % (5.6052 %), Cr- 4.2227 % (4.2242 %) and Mo-46.217 % (46.2109 %).

2.2.2 IR spectrum:

The peaks at 945, 915 and 643 cm^{-1} corresponding to Mo-O_d, Mo-O_b-Mo, Mo-O_c-Mo stretching motion of Anderson structure. The broad peak centered at 3139 cm^{-1} and sharp peak at 1619 cm^{-1} indicates water associated with Anderson structure as either in lattice or in the co-ordinated form.

2.3 General procedure for the oxidation of thiols

To a solution containing Hexaamolybdochromate (III) (2 mol %) and thiol (2 mmol) in 10 mL 50% acetonitrile (v/v), 5 mmol (0.56 mL) of 30% H_2O_2 was added dropwise. The mixture was stirred at 60^0C. TLC is used to monitor the progress of reaction. After completion of the reaction, the solid product was filtered off and recrystallized. While in the case of liquid disulfides, after completion of the reaction the excess H_2O_2 was destroyed by 2 mL of $3x10^{-3}$ M sodium sulphite. The mixture was treated with dichloromethane (2 x 25 mL). The organic layer was dried on anhydrous $MgSO_4$ and it was concentrated to get the required product. The M.P./B. P. were taken and confirmed with theoretical standards.

III. Results and Discussion

Thiophenol was taken as a model compound and the reaction conditions were optimized by varying the solvent, concentration of the catalyst and the oxidant. In acetonitrile, the reaction was completed within 30 minutes. Other solvents provide moderate yields with longer reaction times, while in water there was no reaction even after 24 hours. The effect of catalyst and oxidant concentration was also studied. It has been observed that the reaction affords good yield of product at 2 mol% catalyst and 5 mmol of H_2O_2 concentration. The optimized conditions were used for conversion of series of aromatic and aliphatic thiols to corresponding disulfides in good yields (Table 1). After completion of the reaction the reaction mixture was filtered, and remaining mixture was treated with dichloromethane.

Table 1 Oxidation of thiols by H_2O_2

Entry	R	Reaction time (min)	Yield[b] (%)	M.P./B.P. (^{0}C)
1	C_6H_5	30	84	62
2	FC_6H_5	18	86	46
3	ClC_6H_5	20	94	72
4	BrC_6H_5	18	86	90
5	$CH_3(CH_2)_3$	50	80	117-118
6	$CH_3C_6H_5$	40	83	45

[b]- isolated yields

It has been observed that under experimental condition the uncatalyzed reaction is slow. Also, the rate of the reaction depends on the catalyst and oxidant concentrations. The catalyst and oxidant interact, and reaction gets proceeded.

IV. Conclusions

In summary, we report a mild, efficient, clean method for oxidation of thiols to corresponding disulfides using 30% H_2O_2catalyzed by Andersone type hexamolybdochromate(III). The procedure is attractive due to its simplicity and simple workup procedure.

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Studies on Structural properties of nano-structured $ZnAl_2O_4$ synthesized by sol-gel auto-combustion method

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ABSTRACT: *0.2, 0.3, 0.4, 0.5 and y = 0.5) were synthesised by sol-gel auto combustion method using precursors zinc ni.Nano-structured mixed metal oxides with chemical formula $Zn_x(Al_2)_yO_4$ (Where x = 0.1, tratehexahydrate, aluminium nitrate and glycine. Resulting product of oxides were sintered at 800°C for 4 hours. X-Ray diffraction (XRD), Field Emission Scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR) and Energy dispersive X-ray analysis (EDAX) were used to characterize the materials. The phase purity of sample with x = 0.2 was confirmed by TG-DTA analysis. XRD study shows the formation of cubic spinel structure.*

Keywords: *$ZnAl_2O_4$, Auto-combustion, XRD, FTIR, FESEM, EDAX*

I. Introduction

Sensing and observing of toxic and harmful gases have become the issue of interest for protection and safety of the environment **[1].** $ZnAl_2O_4$ is possessing high thermal stability, low surface acidity, high surface area and high mechanical resistance and hence used as an active constituent for catalysts and it supports for other metal oxides and dispersed metals **[2-4].** Due to these properties, zinc aluminate ($ZnAl_2O_4$) can be used as an ultraviolet photoelectric material as well as optical and electrical coating purpose. Therefore $ZnAl_2O_4$ material has been attracting the attention of many researchers.

Zinc aluminate is a natural mineral basically called as gahnite having normal spinel structure. The general chemical formula for aluminate is shown in the form of parentheses ( ) and square [ ] brackets as $(M)[N]_2O_4$, where M refers to as divalent and N refers to as trivalent metal ions. The parentheses ( ) represents tetrahedral site (A) and square [ ] represents octahedral site (B) within the close- packed FCC unit cell with Fd3m space group symmetry. In normal spinel $ZnAl_2O_4$ structure, Zn^{2+} occupies on A site whereas Al^{3+} occupies on B- site **[5]**.

Many researchers prepared $ZnAl_2O_4$ by various physical and chemical methods like solid-state reaction **[6],** sol–gel process **[7],** reverse micro-emulsion synthesis **[8],** co-precipitation **[9],** hydrothermal synthesis **[10]** etc. Among all these methods sol-gel auto combustion method is convenient, environment friendly, inexpensive and efficient preparation method and provides larger surface area and pore volume with a uniform pore size distribution.

Alison et al.**[11]** synthesized $ZnAl_2O_4$ by sol-gel method and reported that single phase spinel crystal structured zinc aluminate can be successfully prepared at lower temperature. Kapse**[1]** prepared nanocrystalline spinel type metal oxide materials by citrated sol-gel method and studied its gas sensing properties.Structural properties of $ZnAl_2O_4$ films prepared by sol-gel method were reported by Yiquan et al.**[12].** Baochang et al.**[13]** fabricated $ZnAl_2O_4$ for humidity sensors. Guan et al.**[14]** synthesized ZnO/ $ZnAl_2O_4$ materials for ethanol sensing. Vijaya et.al.**[15]** reported humidity sensing characteristics of Sr(II)-added $ZnAl_2O_4$ composites. They reported that the sensing response increases with increase in strontium. The structural properties of $ZnAl_2O_4$ and $NiAl_2O_4$ prepared by citrate precursor method have been reported by Singh et al.**[16].** Tshabalala et al.**[17]** studied the Luminescence properties of $Ce^{3+}$and Tb^{3+} co-activated $ZnAl_2O_4$ phosphor. They reported that cathodoluminescence intensity was stable after 10 hr of electron beam irradiation. Ciupina et al.[18] synthesized $ZnAl_2O_4$ nanocrystals by co-precipitation and micro-emulsion methods and compared their structural properties. It is reported that $ZnAl_2O_4$ synthesized by sol-gel method yields good material as compared to that prepared by co-precipitation and wet mixing methods **[19]**.

In this paper, we are reporting structural properties of zinc aluminate synthesized by sol-gel auto combustion method.

II. Experimental

2.1. Synthesis of $ZnAl_2O_4$

Nano-structured $ZnAl_2O_4$ powder with compositions $Zn_x(Al_2)_yO_4$ (Where x = 0.1,0.2,0.3,0.4,0.5 and

y = 0.5) were synthesized by sol-gel auto combustion method using glycine as a fuel. The required stoichiometric zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$], aluminium nitrate nonahydrate [$Al(NO_3)_3.9H_2O$] and glycine [C_2H_5NO] were dissolved in double distilled water with vigorous stirring. The pH of the solution was then maintained at about 7 by adding drop wise ammonia, thereby occurring a homogeneous clear solution. The solution was constantly heated on hot plate until the complete the process of evaporation and convert it into gel. The resulting gel was then ignited to obtain powder. This powder was milled in agate mortal and sintered at 800°C for 4 hours under static air in muffle furnace. The TG-DTA, XRD, FESEM, EDAX and FTIR techniques were used to characterize the sintered powder.

TG-DTA of mixed metal oxide was carried out using instrument SDT Q600 V20.9 Build 20. The X-ray diffraction patterns of all the metal oxides were recorded on Phillips make model PW3710 with Cu-Kα radiation (λ = 1.5406Å). The surface topography and microstructure of the sample was examined by means of electron microscopy scanned with different magnifications (JEOL JSM-6360). The qualitative and quantitative determination of required elements in the investigated oxides was observed by using energy dispersive X-ray spectroscopy (JEOL JSM-6360). JASCO FT/IR-6100 type 'A' spectrometer was used for FT-IR study of oxide material.

III. Results and Discussions:

3.1. TGA-DTA Analysis:

TG-DTA analysis was performed at a heating rate of 10 °C/min. from room temp to 1000 °C in air atmosphere to investigate the thermal properties of $ZnAl_2O_4$. Typical TGA-DTA curve for the $Zn_{0.2}(Al_2)_{0.5}O_4$ is shown in the Fig. 1. From this figure, it is seen that weight loss of the material take place up to 517.05^{0}C. It means that removal of absorbed water and decomposition of organic residues completed up to 517.05^{0}C . Beyond this temperature, there is no any change observed in weight loss. The DTA curve shows exothermic peak at 517.05 °C indicating the formation of pure $Zn_{0.2}(Al_2)_{0.5}O_4$.

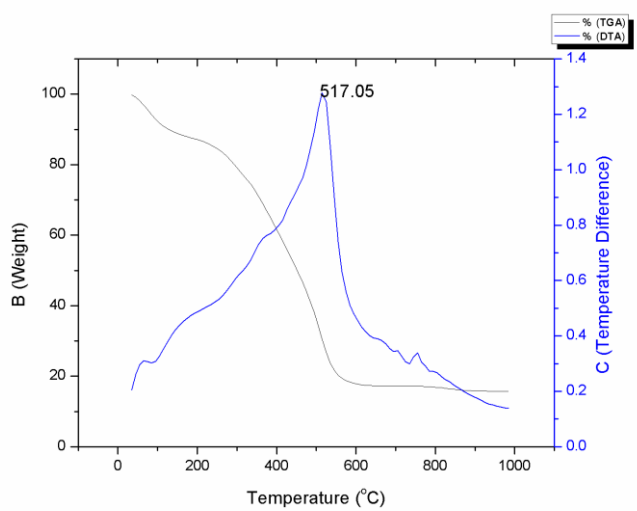

Fig. 1.TG-DTA curves of $Zn_{0.2}(Al_2)_{0.5}O_4$

Ianos et al. **[20]** prepared $ZnAl_2O_4$ by combustion method and they observed significant mass loss at 500 ^{0}C. Rakesh et al. **[16]** synthesized $ZnAl_2O_4$ and $NiAl_2O_4$ spinels by cirateprecusor method. They observed that the decomposition of sample was completed at about 430 ^{0}C .

3.2 XRD Analysis:-

X –ray diffraction patterns of $Zn_x(Al_2)_yO_4$ (Where x = 0.1, 0.2, 0.3, 0.4, 0.5 and y = 0.5) nano-structured oxides are shown in Fig.2. From this figure, it is observed that the intensity of the peaks increases with increase in zinc content up to x = 0.3 and thereafter slightly decreases. It is also seen that intensity of the some additional peaks corresponding to the angles 2θ = 34.59 ^{0}C and 63.05 ^{0}C indicated by the star (*) in the patterns increases for the $x \geq 0.3$ compositions. This may be due to presence of more concentration of zinc oxide. Jain et .al [21] synthesized $ZnAl_2O_4$nano-particles by solution combustion method and observed such an additional peak at 2θ = 34.5 ^{0}C.

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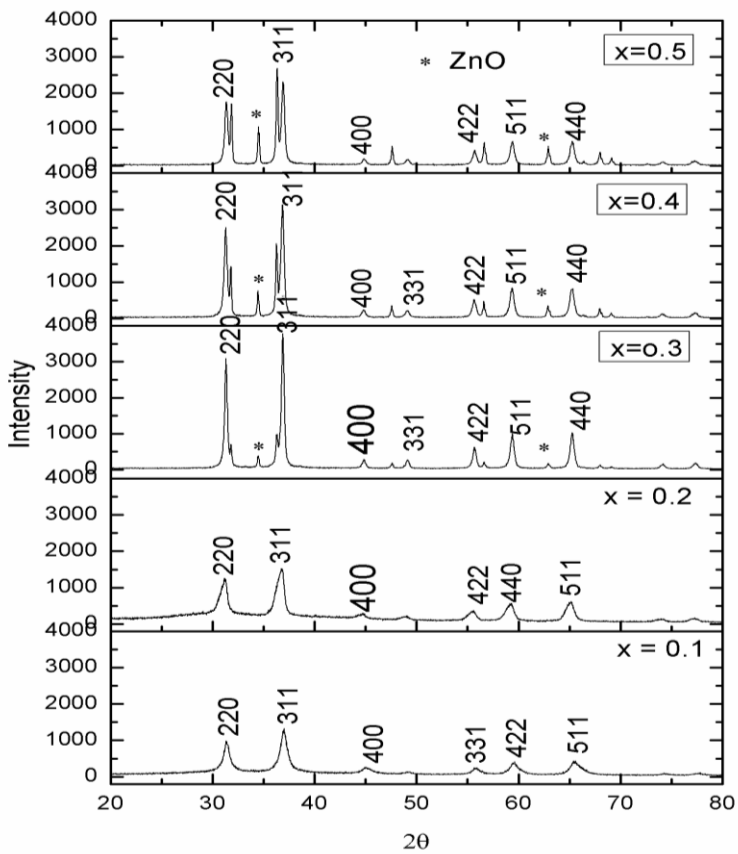


Fig. 2:X–ray diffraction patterns of $Zn_x(Al_2)_yO_4$ (where x = 0.1,0.2,0.3,0.4,0.5 and y= 0.5)

Allowed peaks in the diffraction patterns are in good agreement with JCPD card No. 71-0968. It confirms the formation of cubic spinel structure. The interplanar distances (d_{cal}) is calculated by using relation

$$d_{cal.} = \frac{n\lambda}{2\sin\theta}$$

Where n- order of diffraction
λ-wavelength of X-ray
θ-diffraction angle

The calculated and standard inter-planner distances (d_{cal} and d_{std}) for the composition x = 0.2 are presented in Table 1. It is clear that d_{cal} and d_{std} values are well agree with each other and which also confirms the exact indexing of the hkl planes.

Lattice constant (a) for most intense plane (311) is calculated by using formula [Cullity Bernard Dennis. "Elements of X-ray Diffraction." (1978)].

$$a = d_{cal}\sqrt{h^2 + k^2 + l^2}$$

Table 1 : Inter- planner distances (d_{Cal} and d_{std}) for $Zn_xAl_2O_4$ (x=0.2)

hkl planes	Inter-planner distances (A°)	
	d_{Cal}	d_{Cal} (JCPD card no-71-0968)
220	2.8566	2.88
311	2.4372	2.34
400	2.0215	2.00
331	1.8568	1.83

422	1.6547	1.66
511	1.5610	1.52
440	1.4327	1.49
620	1.2794	1.29
533	1.2355	1.26

Table 2: Lattice constant, crystallite size and grain size of $Zn_x(Al_2)_yO_4$ system

Composition x	Lattice constant (A°)	Crystallite size (nm)	Grain Size (nm)
0.1	7.979	12.05	59
0.2	8.083	9.69	33
0.3	8.065	23.02	41
0.4	8.060	22.79	48
0.5	8.167	33.23	34

The average crystallite size (D) of the mixed metal oxides was estimated for (311) X-ray diffraction peak using Scherrer's formula

$$D = \frac{0.91\lambda}{\beta\cos\theta}$$

Where

λ-wavelength of X-ray
β- Full width of half maxima
θ-Diffraction angle

Lattice constant and crystallite size of all the mixed metal oxides are tabulated in Table 2. From table 2, it is seen that there is no any remarkable trend found for the lattice constant as well as crystallite size of the $Zn_x(Al_2)_yO_4$ system with increasing zinc content. The value of lattice constant for $Zn_{0.2}(Al_2)0.5O_4$ is 8.083 Å and is nearly same as that reported value [JCPD card No. 71-0968].

3.3 FT-IR Analysis :

FTIR spectra of $Zn_x(Al_2)_yO_4$ where x=0.1,0.2,0.3,0.4,0.5 and y=0.5 mixed metal oxides are shown in the Fig. 3. Strong and characteristics bands for inorganic materials are present at far IR region. The bands corresponding to 1000-400 cm^{-1} are related to Al-O bonds. In two different ways the aluminiumcation might coordinate to oxygen namely hexa coordination and tetrahedral coordination. If the cation is hexa coordinated (AlO_6), the Al-O stretching is expected within the range of 500-700 cm^{-1} and bending modes are expected within 330-450 cm^{-1} **[22].** However, a tetrahedral coordination (AlO_4) is expected to give stretching modes within the narrow range 700-850 cm^{-1} and bending modes between 250 and 320 cm^{-1} **[23].**Weaker bands at higher wave number i.e. ~3441 cm^{-1},~3451 cm^{-1}, ~3437 cm^{-1}, ~3452 cm^{-1}, and ~3431 cm^{-1} are because of free O-H stretching vibrations of water molecules adsorbed on surface of samples as reported by **[24].** The band obtained ~ 1628 cm^{-1} is due to water bending as reported by Ianos et al. **[20]. They** reported that the band at 3430 cm^{-1} is due to the stretching vibration of water molecules and the band at 1640 cm^{-1} is due to water bending. These bands signify intermolecular H-bonding as reported by Jain et al. **[21].**Syafiq et al.**[25] and** Jamal **[26]** found a broad absorption band at 3,410 cm^{-1} which corresponds to OH group where it can be identified as water content attributed to the stretching vibrations of water molecules. Absorption bands at ~2343 cm-1, ~2345 cm-1, ~2346 cm-1 is of O=C=O asymmetric stretching vibration of CO_2 which is present in atmosphere **[21].** Strong absorption peaks at ~666 cm^{-1} and ~554 cm^{-1} are of Al-O stretching vibration and O-Al-O bending vibration resply**[27]** confirming the octahedral co-ordination of Al ions and hence formation of normal spinel $ZnAl_2O_4$.

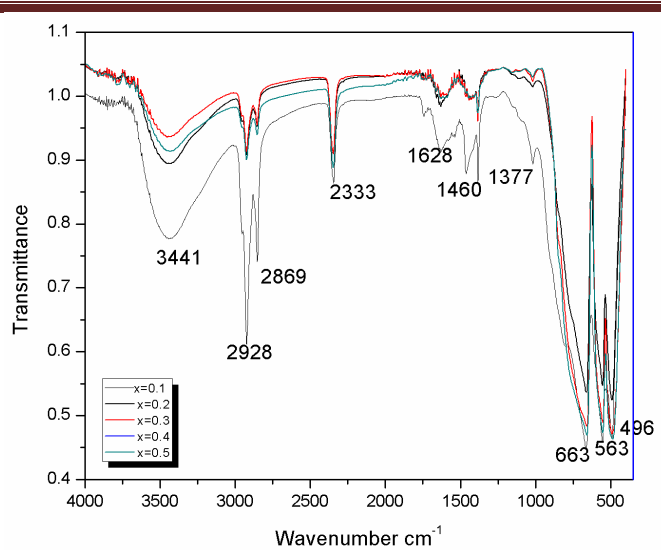
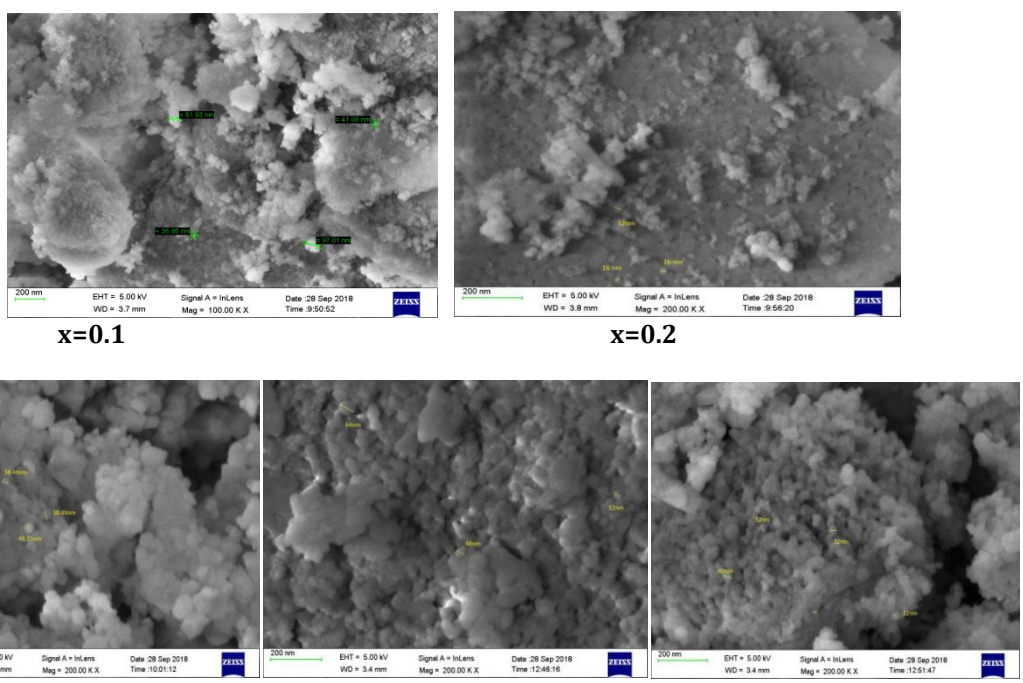


Fig. 3 FT-IR spectra of $Zn_x(Al_2)_yO_4$ (where x = 0.1,0.2,0.3,0.4,0.5 and y = 0.5) system

3.4 FESEM Analysis

The FESEMs of $Zn_x(Al_2)_yO_4$ (x=0.1, 0.2, 0.3, 0.4, 0.5 and y= 0.5) are shown in fig.4



x=0.1 **x=0.2**

x=0.3 **x=0.4** **x=0.5**

Fig. 4 FESEM of $Zn_x(Al_2)_yO_4$ (where x = 0.1,0.2,0.3,0.4,0.5 and y = 0.5) system

From microphotographs, it is seen that spherical grains are formed for all the oxides. The average grain size for all the oxides are calculated by using linear intercept method and presented in table 2. From this table it is seen that as the zinc content, grain size increases except for the composition x = 0.5.

3.5. EDAX Analysis:

X-ray energy dispersive (EDAX) was used to analysis and identify the weight percentage of the element in a $ZnAl_2O_4$ compound by qualitative. EDAX spectrum (Fig.5) indicates the stoichiometric existence of elements Zn, Al and O and the statistic analysis confirms the formation of $ZnAl_2O_4$ phase as shown in table 3

Table 3: Elements content with different compositions of x for $Zn_x(Al_2)_yO_4$ compound

Sr. No.	Composition,x	Element (at.%)			Weight (%)		
		Zn%	Al%	O%	Zn%	Al%	O%
1	0.1	5.67	27.85	66.48	16.96	34.38	48.66
2	0.2	12.42	25.63	61.95	32.55	27.72	39.73
3	0.3	14.17	23.81	62.02	36.16	25.09	38.75
4	0.4	15.04	23.75	61.22	37.76	24.62	37.63
5	0.5	17.47	22.29	60.24	42.18	22.22	35.60

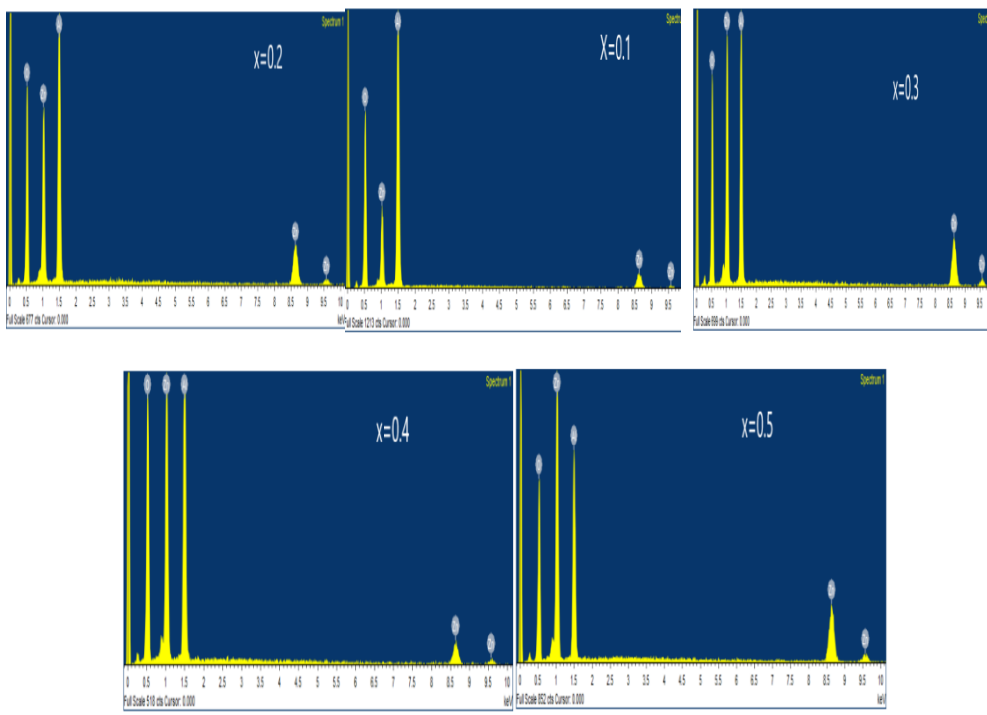


Fig. 5:EDAX of $Zn_x(Al_2)_yO_4$ (where x = 0.1,0.2,0.3,0.4,0.5 and y = 0.5) system

IV. Conclusion:

Zinc aluminates by varying zinc concentration were obtained from sol-gel auto combustion method and investigated their structural properties. The DTA curves shows exothermic peak at 517.05^{0}C indicating the formation of $ZnAl_2O_4$spinels. From XRD it is seen that there is no any remarkable trend found for the lattice constant as well as crystallite size of the $Zn_x(Al_2)_yO_4$ system with increasing zinc content and calculated value of lattice constant for $Zn_{0.2}(Al_2)0.5O_4$ is 8.083 Å which is nearly same as that reported value. The vibrational stretching frequencies corresponding to the composites were confirmed by FT-IR spectroscopy. FESEM microphotographs reveals that as zinc concentration increases there is clear nanosize grain formation. EDAX analysis confirms the presence of only $ZnAl_2O_4$materials without any impurity.

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Synthesis of tetrahydrobenzo[b]pyran using Glycerol as Green Solvent

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ABSTRACT: *An efficient and simple synthesis of some tetrahydrobenzo[b]pyranderivatives was carried out by using three component reactions of aldehyde, ethyl cyanoacetate, dimedone and catalytic amount of CAN (Cerric Ammonium Nitrate) in glycerol as green solventat room temperature. In this work, we have synthesized pyran derivatives using variety of aldehydes. The synthesized compounds are characterized by ^{1}H NMR and IR spectroscopy. The synthesized compounds are screened for their antibacterial activity.*

Keywords: *pyran derivatives, three component reactions, CAN, antibacterial activity.*

I. Introduction

Multi-component reactions (MCRs) are emerging as important tools for efficient synthesis of a wide variety of organic molecules[1]. Such reactions offer a number of practical, environmental, and economical advantages for construction of complex molecular architectures over the more traditional approaches. While numerous MCRs have been devised, relatively few processes that result in the formation of two or more intermolecular carbon–carbon bonds are known.

Unsaturated benzopyran derivatives, generally known as chromenes, form the fundamental unit of many biologically active natural products as well as synthetic therapeutic agents. The chromene nucleus is generally regarded as a privileged skeleton in medicinal chemistry. Due to oxygen atom presence in the structure, chromenes acquires broad pharmaceutical significance[2]. Achievement in synthesis of chromine nucleus from pyran is of highest interest.Pyran derivatives are one of the most common compounds present in various biologically active natural and synthetic products[3]. Polyfunctionalized 4H-pyran, a major constituent of many natural products[4-6], is known for its wide array of biological activities such as antitumor, antibacterial, antiviral, spasmolytic, and antianaphylaxis[7-10]. Recent findings have suggested that the compounds having 4H-pyran core are useful for the treatment of Alzheimer, Schizophrenia, and Myoclonus diseases[11].Giventheimportantproperties possessed by Polyfunctionalized 4H-pyrans, it is natural to havemany synthetic endeavorstoachievethetargetbyadopting simple reaction strategies.

Moreover, 4H-pyran derivatives are considered as potential calcium channel antagonists[12] because their structure is similar to that of the biologically active 1, 4-dihydropyridines. Polyfunctionalized 4H-pyrans have been received considerable attention due to their wide range of biological activities. The pyran derivatives exhibit important pharmacological properties such as antimicrobial and antioxidant agents. Apart from their own biological activities, the 4H-pyrans can serve as useful intermediates for the synthesis of various heterocyclic compounds, such as the pyrano[2,3-b]pyridine derivatives, polyazanaphthalenes, pyrano[2,3-c]pyrazoles, pyrano[2,3-d]pyrimidines[13,14] and pyridin-2-ones[15,16]which are also potentially biologically active.

In continuation of our investigations on the development of new synthetic methodology, we herein report an innovative, convenient, mild and efficient procedure for the synthesis of pyrans. One-pot three component reaction of aldehyde derivatives with ethyl cyanoacetate, dimedone and catalytic amount of CAN were stirred at room temperature utilized for synthesizing of pyrans. The experiments carried out using different quantities of glycerol at ambient temperatures

II. Experimental:

All organic materials were purchased commercially from SDfine and Merck and were used without further purification.

All products were characterized by MP, IR and 1HNMR. Melting points were measured using a fine control Electro thermal capillary apparatus make Equptronicsand are uncorrected. ^{1}HNMR spectra were recorded on Bruker DPX spectrometer at 300MHz NMR spectra were obtained on solutions in $CDCL_3$ Chemical shifts are reported imparts of million (δ.ppm) relative to TMS (δ.0.0) as internal standard. IR spectra were obtained as potassium bromide (KBr) pellets with a Lambda Scientific FT IR-7600PCspectrometer. Thin layer chromatography (TLC) on commercial aluminum-backed plates of silica gel to monitor the progress of reactions.

2.1 General procedure for Synthesis of 4H-pyran derivatives (4a-l)

A solution of an aldehyde **1** (10 mmol), Dimedone**2** (10 mmol), ethylcyanoacetate**3** (10 mmol) and CAN*(Cerric Ammonium Nitrate)*(10 mol %) was stirred in glycerol (15 ml) (with different volumes) at room temperature. After completion of the reaction, as indicated by TLC, the product obtained was poured in ice cold water. The solid product was filtrated and then consequentially washed with water, and dried. Then recrystallized with acetone to obtain pure product. The physical data (NMR, and IR) of these known compounds were found to be identical with those reported in the literature.

2.2Spectral Data:

Ethyl 2-amino-4-(4-methoxy phenyl)-7, 7-dimethyl-5-oxo 5,6,7,8 tetra hydro benzo [4H] pyran-3-carboxyl ate.(4a): Pale yellow ; mp 188-190°C; IR (KBr) (υ_{max}, cm^{-1}): 1594.84 (C=O), 1245.79 (C-O), 1169.06(-OMe); ^{1}H NMR(300 MHz,$CDCl_3$): δ_H (ppm) 0.98 (3 H, s, Me), 1.13 (3 H, s, Me), 1.42 (3 H, t, CH_3), 2.12 (1 H, d), 2.22 (1 H, d), 2.49 (2H, s), 3.98 (3 H, s, OCH_3), 4.41 (2 H, q, OCH_2), 4.80 (1 H, s), 7.36 (2 H, d), 7.85 (2 H, d).

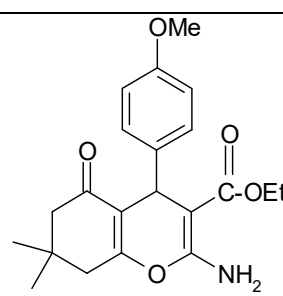
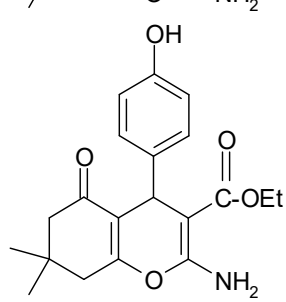
Ethyl 2-amino-4-(4- hydroxy phenyl)-7, 7-dimethyl-5-oxo 5,6,7,8 tetra hydro benzo [4H] pyran-3-carboxyl ate.(4b): White powder; mp142^{o}C;IR(KBr) (υ_{max}, cm^{-1})1595.84 (C=O), 1249.65 (C-O) 1H NMR (300 MHz, $CDCL_3$):δ_H (ppm) δ 9.30 (s,1H, OH),6.96–6.89 (m, 4H), 6.67 (d, 2H), 4.07 (s, 1H), 2.55–2.43 (m,2H), 2.24 (d, 1H), 2.09 (d, 1H), 1.03 (s, 3H), 0.95(s, 3H), 1.40 (3 H, t, CH_3) 4.10(q, 2H, CH3CH2).

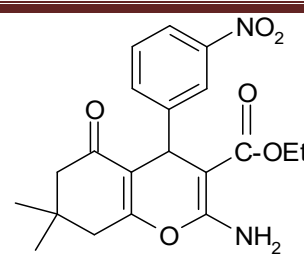
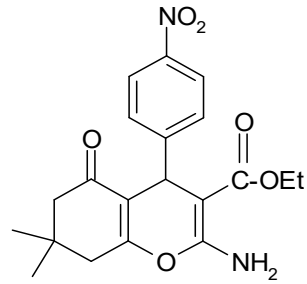
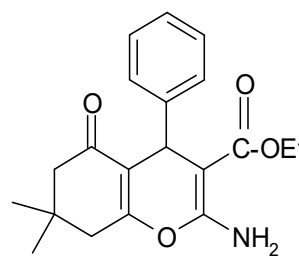
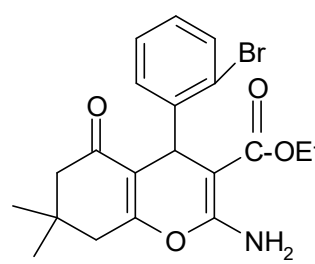
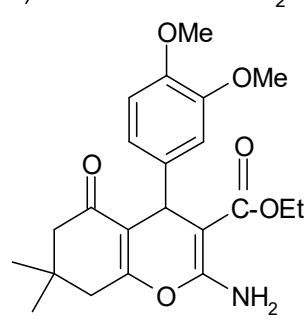
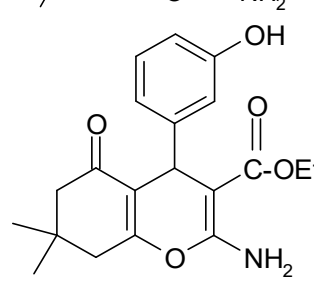
Ethyl 2-amino-4-(4-nitro phenyl)-7, 7-dimethyl-5-oxo 5,6,7,8 tetra hydro benzo [4H] pyran-3-carboxyl ate. (4d): Yellow powder; mp180-182°C;IR (KBr) (υ_{max}, cm^{-1})1587.13 (C=O), 1245 (C-O), 1365.35 (NO_2).1H NMR(300 MHz,$CDCL_3$): δ_H (ppm) δ 8.161 (d, 2H), 7.432 (d, 2H), 7.263 (s, 2H), 4.383 (s, 1H), 2.571(s, 2H), 2.250 (d, 1H), 2.085 (d, 1H), 1.063 (s, 3H), 0.986 (s, 3H),7.82(S,2H, NH_2), 1.05(t, 3H,CH_3), 4.14(q, 2H, CH_2).

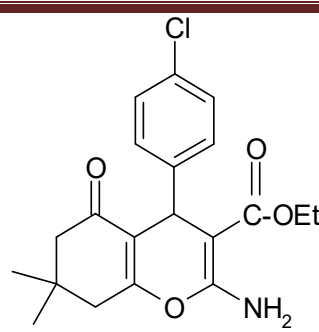
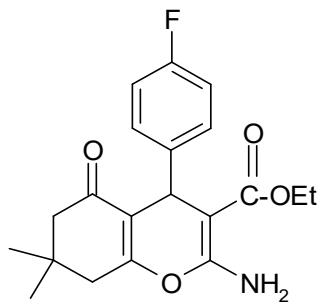
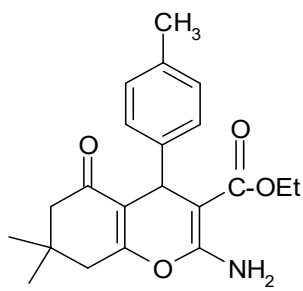
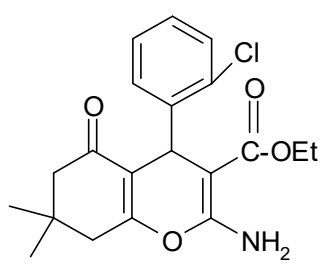
Ethyl 2-amino-4-(4 phenyl)-7, 7-dimethyl-5-oxo 5,6,7,8 tetra hydro benzo [4H] pyran-3- carboxyl ate. (4e): Yellow powder; mp156-158^{o}C; IR (KBr) (υ_{max}, cm^{-1})1596.59 (C=O), 1245 (C-O); 1H NMR (300 MHz, $CDCL_3$):δ_H (ppm) δ 7.146-7.314 (m, 5H), 6.998 (s, 2H), 4.189 (s, 1H), 2.526 (s, 2H), 2.264 (d, 2H), 2.115 (d, 2H), 1.050 (s, 3H), 0.967 (s, 3H), 7.80(S,2H, NH_2), 1.32(t, 3H,CH_3), 4.44(q, 2H, CH_2).

Ethyl 2-amino-4-(2- bromo phenyl)-7, 7-dimethyl-5-oxo 5,6,7,8 tetra hydro benzo [4H] pyran-3-carboxyl ate. (4f): White powder. Mp210^{o}C; IR (KBr) (υ_{max}, cm^{-1}) 1591.17 (C=O), 1248.72 (C-O), 695.18 (C-Br); 1H NMR (300 MHz, DMSO-d6):δ_H (ppm) d 1.11–1.18 (m, 6H, 2CH3), 2.23 (s, 3H), 3.65–3.85 (m, 4H, CH2), 4.82 (s, 1H, CH), 6.91 (s, 2H, NH2), 7.43 (d, 2H, ArH), 7.50 (d, 2H, ArH), 1.05 (t, 3H,CH_3), 4.64(q, 2H, CH_2).

Table 1: Synthesis of Ethyl 2-amino-4-(phenyl)-7, 7-dimethyl-5-oxo 5,6,7,8 tetra hydro benzo [4H] pyran-3- carboxylate:

Entry	R	Product	Time min	Yield %	mp found (^{0}C)	mp reported (ref) (^{0}C)
4a	4-OCH_3		120	87	188-190	188-190[19]
4b	4-OH		90	89	142	-

4c	3- NO_2		90	**91**	183-184	182-183[17]
4d	4- NO_2		90	91	180-182	184[18]
4e	H		75	89	156-158	155-158[17]
4f	2-Br		75	92	210	-
4g	3, 4-OCH_3		120	86	157	155-157[21]
4h	3-OH		90	88	182	180-183[22]

4i	4-Cl		75	92	154-156	157[18]
4j	4-F		90	91	155	152-155[20]
4k	4- CH_3		120	90	157-159	155-156[18]
4l	2- Cl		75	90	180-182	183-185[17]

III. Result and Discussion:

In a preliminarily investigation on the model reaction of 4-chlorobenzaldehyde, dimedone, Ethyl cyano acetate and CAN (cerric ammonium nitrate) as catalyst is chosen. The excellent yield found when glycerol used as solvent. Model reaction was carried out with different amount of the same. With 10 mmol of each reactant, reaction with 5, 10, 15 and 20 mL of glycerol was tried and it was found that 15mL of solvent is sufficient to get the product in good yield (Table 1). Increase in quantity of catalyst and solvent did not show any change on yield and reaction time. Thus, 15 mL of glycerol and CAN (10 mol %) was chosen as optimum amount to catalyze the reaction.

Table 2: Optimization of amount of solvent:

Sr. No.	Amount of Solvent (mL)	Time (min)	Yield %
1	5	150	55
2	10	165	50
3	15	120	90
4	20	150	75

After optimization of the reaction conditions, to explore the efficiency and the scope of presented protocol, dimedone (10 mmol) and ethyl cyano acetate (10 mmol) were treated with structurally diverse aromatic aldehydes (10 mmol) and CAN (10 mole %)as catalyst in the presence of glycerol as solvent. The corresponding results are summarized in **Table 1**. As Table2 indicates, all aldehydes (including benzaldehyde and aryl aldehydes bearing halogens, electron-withdrawing as well as donating substituents) were successfully reacted to produce the corresponding pyran derivatives in good to excellent yields and in relatively short reaction times. The presented method was successfully used for aryl aldehydes with various groups at different positions such as halides, nitro and hydroxyl.

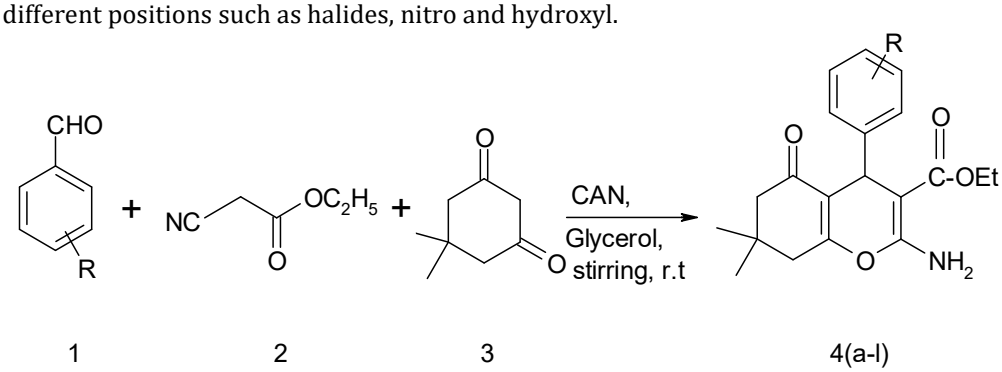


Fig1: Scheme for synthesis of tetrahydrobenzo[b]pyran derivatives.

3.1 Antibacterial activity (Agar Well method):

In this method, different dilutions of antibiotic bored or other fermentation products are placed inside the wells that are bored on the surface of agar medium and from these wells the compound diffuses into the surrounding area.

Sterile Petri plates are poured aseptically with about 15-20 ml of suitable, sterile, nutrient agar medium and sauboraud'sagar.This medium is allowed to solidify. 0.1 ml of suspension of test organisms E. coli (gram negative bacteria), Staph.aureous, Bacillus Subtitles (gram positive bacteria)etc.(Sauboraud's agar) were spreaded with use of sterile glass spreader.Then with use of sterile borer, wells were created on the agar plates. 5µg/ml of the test compounds dissolved in suitable solvent was then added in this wells. Along with test compound, the solvents used to dissolve, was also added in well as standard. The plates are incubated at 37^0C temperature for 24 hours. After incubation, the zone diameters of inhibition of growth of test organism are measured for each of the test compounds and standard of solvents.

The zone inhibition shown in table no 3. The compound (4d) showed better inhibition against S. aureus, E.coliandBacillus subtitles. Compound (4j) showed better inhibition against S. aureus, and Bacillus subtitles. While showed no inhibition against E.coli. Compounds (4a, 4e, 4i, 4j) showed no inhibition against E. coli.while are moderate active against S. aureus, and Bacillus subtitles. Compound 4c showed less inhibition against S. aureus, E.coli and Bacillus subtitles. Overall all the compounds (4a-4j) show antibacterial acivity in some way or another.

Table 3: Antibacterial activity zone of inhibition:

Sr. No	Compound	Antibacterial activity Zone of inhibition in (mm)			Antibacterial activity*
		S. aureus	E.coli	Bacillus subtitles	µg/ml
1	4a	20	-	20	++
2	4b	20	12	30	++
3	4c	15	18	15	+
4	4d	32	24	25	+++
5	4e	25	-	13	++
6	4h	30	12	18	++
7	4i	25	-	13	++
8	4j	30	-	20	+++

*(+Slight antibacterial activity, ++ Moderate antibacterial activity, +++ High antibacterial activity)

IV. Conclusion:

In conclusion, CAN and glycerol can serve as an efficient catalyst and solvent for the synthesis of tetrahydro-benzo[b]pyran.

We described a successful strategy including, an efficient and convenient green synthesis, for the preparation of tetrahydro-benzo[b]pyran derivatives via a three-component reaction between dimedone, aldehydes, active methylene compound using CAN as catalyst. The method offers several advantages including high yield of products, mild reaction conditions, using the inexpensive, nontoxic, and easily available as well as easily isolable catalyst and solventwhich makes it an attractive process for the synthesis of tetrahydro-benzo[b]pyrans.

Results of these study revealed that almost all the synthesized derivatives showed promisingly significant activity on both gram positive and gram negative bacteria and we can conclude that they are antibacterial agents.

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Nanostructured composite in photodegradation of benzidine-derived dye

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ABSTRACT: *Spinel phase mixed-metal oxide is substantially used for heterogeneous photocatalysis of aqueous suspensions of $NiFe_2O_4$ offers an attractive technique that is able to purifying waste water. Dye pollutants from the textile industry are a vital source of environmental contamination. In the present study magnetic and nanosized $NiFe_2O_4$ was prepared by sol-gel technique and its application for photocatalytic degradation of Congo red was studied. Photocatalytic degradation of Congo red was followed under UV irradiation using $NiFe_2O_4$ suspension. The received consequences are encouraging and have sound efficiency of heterogeneous photocatalysis in complete degradation of Congo red (disodium 4-amino-3-[4-[4-(1-amino-4-sulfonato-naphthalene-2-yl) diazenylphenyl] phenyl] diazenyl-naphthalene-1-sulfonate) in aqueous solution.*

Keywords: *Sol-gel, Photocatalysis, Nanocomposite*

I. Introduction

Synthetic dyestuff is one of the major constituent of textile, paper making, printing industries and dye work. The effluent of these industries is highly colored and disposal of these wastes into receiving water can cause damage to surface and ground water of the environment. Photocatalysis is a green technology for treatment of these pollutants in current era [1-7, 8]. Now a days azo (-N=N-) chromophores are extensively used in cloths, leather accessories, furniture and plastic products. Of all dyes approximately 50-70 % dyes are aromatic dye [9, 10]. More than 20% of unconsumed dye enters into the environment [11]. Most of azo dyes and their degradation products such as aromatic amines are highly carcinogenic [12]. Various physical techniques viz. adsorption, biochemical degradation, chlorination, flocculation, reverse osmosis and activated carbon are applied for degradation of dyes [13, 14]. These techniques are not destructive but only transfer the contaminants from one phase to others. During this practice, heterogeneous photocatalysis having advanced oxidation process is found an emerging technology in recent era.

For the same, mostly TiO_2 and doped TiO_2 heterogeneous catalysts are used for degradation of Congo red dye [15-22]. Along with them, ternary metal oxides, containing transition metal ions drawn great attention in the field of photocatalysis. Its semiconducting nature with low band gap, high thermal as well as chemical stability, narrow particle size, high surface area, magneto-resistive properties and low fabrication cost results an better alternative for photocatalysis [23,24]. A lot of researchers had reported the enormous photocatalytic properties of spinel ferrites synthesized by different techniques [25-32].

Our previous published work on the synthesis and properties of nickel ferrite composite materials by simple, less expensive sol-gel auto combustion technique [33]. In continuation this work, prepared updated scale of nickel ferrite composite material, we are main focused on the investigation on physic-chemical characterization and photodegradation of Congo red dye (disodium 4-amino-3-[4-[4-(1-amino-4-sulfonato-naphthalene-2-yl) diazenylphenyl] phenyl] diazenyl-naphthalene-1-sulfonate) in aqueous solution due to its enormous thermal, chemical and physical stability.

II. Experimental

Nickel ferrite ($NiFe_2O_4$) was prepared by sol-gel auto combustion route [33] and characterized by various spectral techniques viz. XRD (Philips PW-1710 using $CuK\alpha_1$ radiations), SEM (JOEL - JSM 6360), TEM (Philips CM-20), Magnetic hysteresis (at RT) and DC electrical resistivity (Two probe technique in the temperature range of 303-773K)

The Photodegradation of Congo red (100 ppm) dye using $NiFe_2O_4$nanaoparticles were assessed under UV region. A 100 mL volume of aqueous solution was used with 100 mg catalyst in whole study. A jacketed quartz tube containing photoreactor having mercury lamp of 125 W was used as photosourse. Self

degradation of CR was followed in same reaction condition in absence of catalyst. The suspension solution was irradiated with continuous stirring in dark for 60 min. to ensure the establishment of adsorption / desorption equilibrium. After certain interval of time 2-3 mL sample was taken out to check the change in concentration. The progress of reaction was monitored using UV-Visible Spectrophotometer. Degradation efficiency was calculated using following equation:

$$\% \text{ degradation} = \frac{C_0 - Ct}{C_0} \times 100 \quad \text{_____ (1)}$$

C_0 =Initial Concentration of CR

Ct = Instant concentration in the sample

III. Result and Discussion

3.1 Structural characterization

The XRD pattern of $NiFe_2O_4$ is shown in Fig. 1.The sharp and intense peaks reveals the well crystalline nature of single phase cubic spinel structure. Crystallite size was calculated by using Scherer's formula with the help of most intense (311) plane and the average crystallite size of the composite materials were found to be 43.7 nm. Lattice constant (a) is 8.341A°. All values are in good agreement with the earlier reported value [33].

3.2 Nanostructure and surface morphology

The surface morphology of nickel ferrite composite used to be additionally studied by means of scanning electron microscopy (SEM). The SEM micrograph of nickel ferrite composite is provided in Fig.2 (a). It's found from scanning electron microscopy (SEM) that the uniform distribution of grains over the surface while agglomerisation of grains is takes place up to certain level. TEM image of $NiFe_2O_4$ is shown in Fig. 2 (b). The average particle size was found that ~ 30 nm, this value is in accordance with XRD results.

3.3 DC electrical resistivity

The temperature dependent dc electrical resistivity measurements in the temperature range 303 – 573 K, as given in Fig. 3, shows that by increasing temperature, resistivity were decreases. It is, therefore, said that these samples have semiconducting nature.

The conduction mechanism in ferrospinels was explained by Verway de-Bohr [34], which involves the exchange of electrons between the ions of same elements having variable oxidation state. The above elements are randomly distributed over different crystallographic lattice e.g. $Fe^{2+} \leftrightarrow Fe^{+3}$, $Ni^{2+} \leftrightarrow Ni^{+3}$. The plots of log ρ vs. $10^3/T$ shows linear nature without any break obeying Wilson's law, $\rho = \rho_0 \exp^{\Delta E/KT}$

Activation energy in eV is 0.42. The calculated values reveals that, conduction is happen in the samples is due to polaron hopping. [33]. The variation in conduction is due to $Ni^{2+} \leftrightarrow Ni^{+3}$ and hopping of electrons from Fe^{2+} to Fe^{+3}.

$$Ni^{2+} + Ni^{+3} \leftrightarrow Fe^{2+} + Fe^{+3}$$

3.4 Magnetic properties

Hysteresis loop of sintered photocatalyst is shown in Fig. 4. Various magnetic parameters like, saturation magnetization, coercive field, and remanent magnetization are 44 emu/gm, 146 Oe and 7.9 emu/gm respectively. The interaction amongst tetrahedral (A) site and octahedral (B) site named A-B interaction gives certain A-B interaction among different ions (Ni^{2+} , Ni^{+3} , $Fe^{2+}$and Fe^{+3}).

3.5 Photocatalytic performance and mechanism

Congo red shows maximum absorption at 498nm. The degradation of dyes is observed as the decrease in absorption of dye solution at regular interval (Fig. 5). The absorption peak became weaker with irradiation time and hence azo group along with aromatic part of dye destructed under UV irradiation. Nickel ferrite displayed enormous photocatalytic performance as almost 99% in the period of 16 hrs. This massive activity is may be due to the effective recombination centers for electron and holes which are avail from'd' orbital's of transition metal ions of catalyst [27, 35-37]. High surface area of catalyst for adsorption of photon and interaction of molecules of reactant with catalyst, due to which number of holes , hydroxyls radicals and super oxides get increased. In addition to that, it's optical, electrical and nanaosize cystallinity, phase purity play vital role in photocatalysis. The mechanism is as follows,

$$\text{Semiconductor} + \text{photons (hv)} \rightarrow e^- (CB) + h^+ (VB) \quad \text{_____ (2)}$$

$$H^+ + H_2O \rightarrow H^+ + OH \quad \text{_____ (3)}$$

$$H^+ + OH^- \rightarrow OH^- \quad \text{_____ (4)}$$

$$e^- + O_2 \rightarrow O_2^{\bullet} \quad \text{_____ (5)}$$

$2e^- + O_2 + H_2O \rightarrow H_2O_2$ _____ (6)

$e^- + H_2O_2 \rightarrow OH^\bullet + OH^-$ _____(7)

The excited electron-hole is responsible for effective results. Nickel ferrite as photon absorbent can strongly absorb and transfer photon energy. The generated electron-hole due to transition metal spinel compounds speed up the rate of reaction. The produced ·OH due to reaction of holes with dye or interaction with hydroxyl group/ H_2O play central role of strong oxidant for photocatalytic process.

Following are the probable steps of degradation,

1 Cleavage of benzene ring (side ring)
2 Cleavage of C-S bond among aromatic ring and sulphonate group by ·OH
3 Cleavage of C-N and C-C bond of chromophores
4 –N=N- double bond cleavage

3.6 Reusability:

The spent catalyst was tested for reusability and stability after separation from reactor by applying magnetic field. Filtered off catalyst is washed by ethanol and dried at 120^0C. In reusability result, its activity is reduced up to 68%. Its structure is intact confirmed by XRD, there is no any addition peak in spectra except decrease in intensity of peak which may be surface reduction of catalyst (Fig. 6).

IV. Conclusion:

The Chemical sol-gel method offers a good alternative material for photocatalysis of Congo red azo dye. Its narrow particle size, single phase , low band gap , optical, surface and magnetic properties along with photoactivity offers effective material for waste water treatment. As area of research directly related to health and environmental bennie, the development in this area will be useful to society and our country.

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Studies on hydrothermal synthesis and characterization of TiO_2nanorods

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ABSTRACT: *In present days, one of the promising nanomaterials for fabrication of the Dye sensitized solar cell (DSSC) is titanium dioxide. TiO_2 has an excellent characteristic to enhance the efficiency of DSSC. Along with this, TiO_2 has a wide range of applications such as photocatalysis, gas sensors, photoelectrolysis, supercapacitors, biosensors, lithium batteries etc. In this paper, we report the synthesis and characterization of rutile TiO_2nanorods which have been synthesized by surfactant-free hydrothermal method. The precursor used in this synthesis is 0.5 ml Titanium (IV) isopropoxide (TTIP) and fluorine doped tin oxide (F: SnO_2) used as a substrate. Hydrothermal temperature is maintained at 140°C for 3 hours. The crystallite size, functional group, surface morphology and elemental composition have been investigated by X-ray diffraction (XRD), FT-IR Spectrometer, and Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) respectively. X-ray diffraction study shows the formation of rutile phase TiO_2. SEM image reveals the formation of nanorods with average length of 3.70 μm.*

Keywords: *TiO_2, Rutile phase, Hydrothermal Method*

I. Introduction

Today, the use of clean energies is essential to contribute to the current demand for energy. Many researchers have devoted much attention to dye-sensitized solar cells (DSSCs) with potentials of high energy conversion efficiency and low fabrication cost. Dye sensitized solar cell (DSSC) play strongly as a next generation solar cell for various applications [1]. Titanium dioxide (TiO_2) is one of the most promising materials used for fabrication of dye-sensitized solar cells (DSSCs) due to its appropriate energy levels, dye adsorption ability, low cost, and easy preparation. TiO_2 is widely used as an active material for Dye sensitized solar cell. Also it is one of the excellent semiconducting materials with wider band gap synthesized for various applications such as photo catalysis [2-3], photoelectrochemical cells [4-5], electrochromic devices [6], gas sensing [7], Li-ion batteries [8] etc. Along with these applications it is also used as a white pigment in cosmetics, paints, papers because of its high refractive index. It is used as opacifier in ceramic glazers. Over the past decades, a large number of synthesis methods have been reported to the preparation of crystalline, size, and shape-tailed nanostructured TiO_2 and it is synthesized by various methods such as Sol gel [9-10], spin coating [11-12],Chemical vapour deposition[13], spray pyrolysis [14], dip coating[15], Solvothermal [16], chemical bath deposition [17], Hydrothermal Method [18-19].

The present research scenario of TiO_2-based DSSCs reports that the efficiency of TiO_2 depends on its crystalline phase, particle size, surface area, etc. There are two major phases of TiO_2 are exists anatase and rutile. Due to the high refractive index rutile phase TiO_2 has excellent light scattering characteristics, which is very important property of the perception of effective light harvesting.

Among all the reported processes, hydrolysis of titanium precursor in the presence of Titanium (IV) isopropoxide is found very high efficiency in the control of the crystalline and the morphology of the nanostructured TiO_2. Hydrothermal method is most promising method to prepare TiO_2 nanomaterial as it is simple and cost effective, requires less temperature and time. This process comprise simple route and produces high crystallinity oxides. In hydrothermal method, by controlling temperature we can control the crystallite size of nanostructures.

This paper report studies on the synthesis of rutile TiO_2nanorods with micro flower morphology by surfactant free single- step hydrothermal route and its characterizations.

II. Experimental details

2.1 TiO_2 thin film deposition

In this hydrothermal synthesis, precursor used for TiO_2 thin film deposition is titanium (IV) isopropoxide (**spectrochem, 98%, $C_{12}H_{28}O_4Ti$**). Fluorine doped tin oxide (FTO) (F: SnO_2) **(~8 Ω/cm, 2.5**

cm × 2.5 cm) used as a substrate. All chemicals in this synthesis are of analytical grade and used without further purification. Double distilled water used as a solvent.

The procedure followed in this synthesis is: 0.5 ml of Titanium (IV) isopropoxide added to equal volume of double distilled water and Conc. HCl (35.40%, Thomas Baker). To get the homogenous transparent solution given mixer stirred for half an hour. The solution obtained after stirring transferred into Teflon lined stainless steel autoclave. In this solution, clean conducting FTO substrate is inserted which is inclined to the wall with conducting side facing upwards. The autoclave is placed in muffle furnace having temperature 140°C for 3 hours. Cool the autoclave naturally. The TiO_2 deposited FTO substrate is then rinsed with double distilled water and dries at room temperature. The deposited TiO_2 thin film sample finally annealed at 450°C for 1 hour. The deposited TiO_2 film is named as T_5.

2.2 Characterization of TiO2 thin films

X-Ray Diffractometer (**RigakuUltimadiffractometer using Cu-Kα radiation)** is used to determine the crystallite size and phase of synthesized sample. To study the functional group PerkinElmer FT-IR Spectrometer is used. Scanning electron microscope/EDS (**FESEM and EDS = JSM-7001F, JEOL, Japan**) is used for surface morphology and elemental composition analysis.

III. Result and Discussion:

3.1 X ray diffraction studies

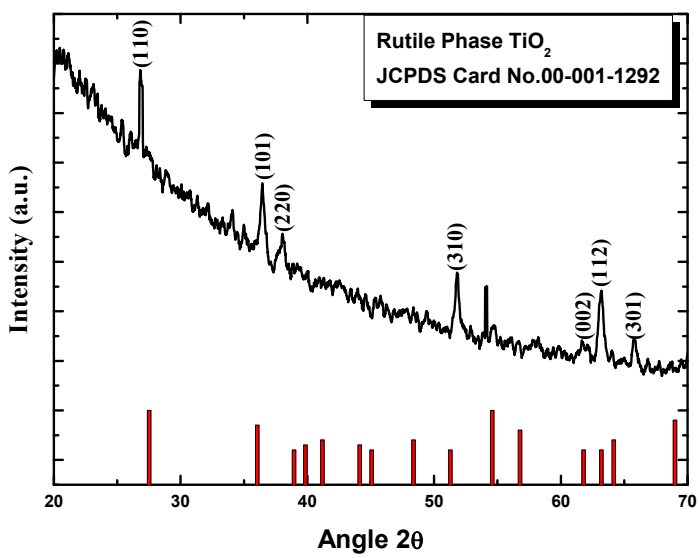

Fig. 1 X ray diffraction pattern of TiO_2 (T_5) sample

XRD patterns of the TiO_2 films deposited on FTO substrate is as shown in figure 1. The calculated 'd' value from the XRD pattern is compared with standard JCPDS data (00-001-1292) shown in figure 1 with red bars. The observed XRD data is well matched with standard JCPDS data which confirms the formation of Rutile TiO2 phase having a tetragonal crystal structure.The lattice parameters a and c for TiO_2 is calculated by using relation given below as,

$$\frac{1}{d^2} = \frac{h^2 + k^2}{a^2} + \frac{l^2}{c^2} \quad \text{---------- (1)}$$

The most intense peak from the observed XRD pattern has the values of lattice parameter and cell volume as, a = 4.69Å, c = 2.51 Å and cell volume = 55.21 $Å^3$ respectively are found to be in good agreement with the standard JCPDS values. The peaks for the anatase and brookite phase are not observed in XRD pattern which shows the high purity of sample.

The Debye Scherer formula is used to find out crystallite size of sample T_5 from X-Ray line width as[20],

$$D = \frac{0.9 \times \lambda}{\beta \cos\theta} \quad \text{---------- (2)}$$

The calculated crystallite size of the sample T_5 from rutile (110) diffraction peak is about 41.90 nm.

3.2 FT-IR studies:

The FT-IR spectraof 0.5 ml TiO_2 sample in the range of 400 cm^{-1} to 2000 cm^{-1} is shown in the figure 2. The sample shows transmittance in the regions at 400-1200 cm-1. The percentage of transmittance is observed at wavenumbers of 421 cm^{-1}, 755 cm^{-1} and 897 cm^{-1}. In figure 2 the strong band in the range between 400 cm^{-1} to 500 cm^{-1} belongs to the characteristic peaks of Ti-O stretching frequencies. For rutile TiO_2 thin film absorbance is seen at ~ 421 cm^{-1} as expected.

From this spectrum, it is also observed that strong band in the range of 1200 and 450 cm^{-1} are apparently associated with the characteristic vibrational modes of TiO_2. The peaks observed at 897 cm^{-1}, 755 cm^{-1} are due to the presence of Ti - O bond.

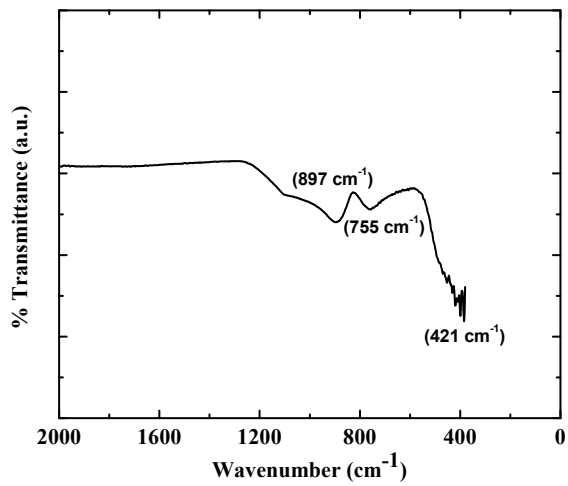

Fig. 2 FT-IR image of rutile TiO_2 (T_5) sample

3.3 Scanning electron microscope studies:

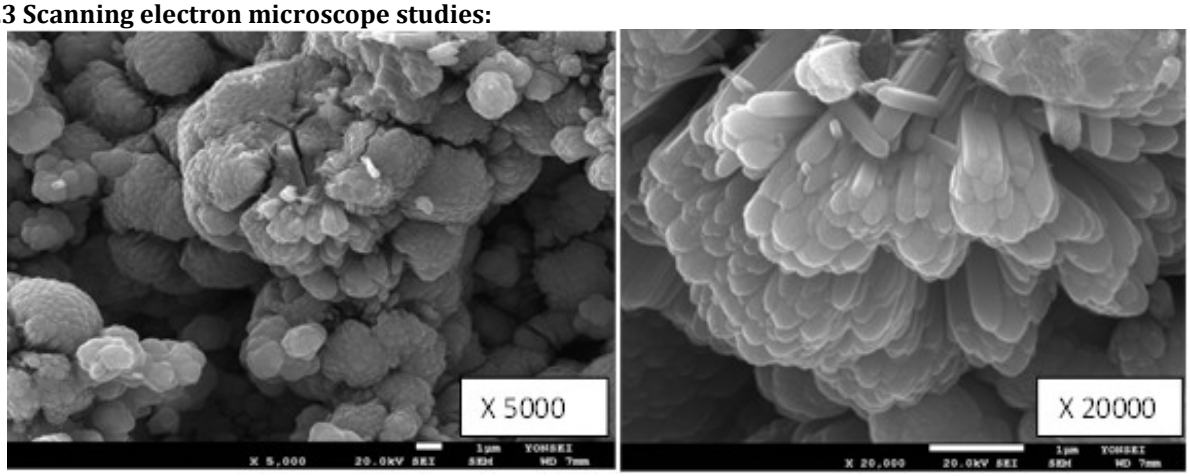

Fig. 3 SEM images of TiO_2 (T_5) sample with x 5000 and x 20000 magnifications

Fig. 3 shows the SEM image of the obtained TiO_2 thin film with x 5000 and x 20000 magnification. The formation of different TiO_2 nanostructures depends on the hydrolysis of titanium (IV) isopropoxide. The rapid hydrolysis of titanium (IV) isopropoxide led to the formation of titanium (IV) complex ions and then the dehydration of titanium (IV) complex ions developed different TiO_2 nanostructures. From the SEM image it is observed that TiO_2nanorods with well-defined micro flowers were formed. The average length of TiO_2nanorods formed is around 3.70 μm. There are numerous concentrically grown TiO_2nanorods, which acts as a nucleation centre for the growth of micro flowers as shown in the figure.

3.4 Elemental compositional analysis:

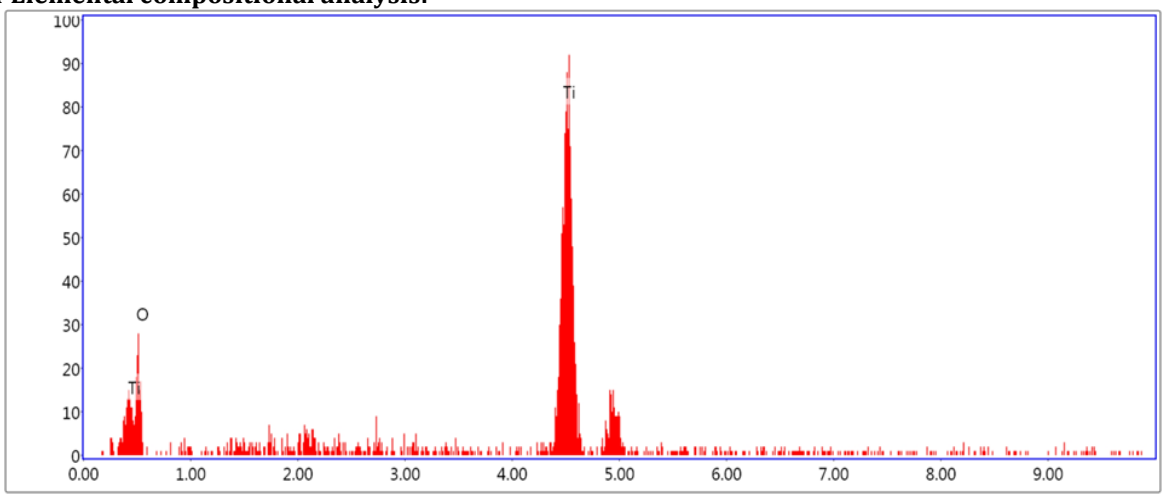

Fig. 4 Energy Dispersive X ray (EDS) spectrum of TiO_2 (T_5) sample

The chemical composition of TiO_2 assessed by energy dispersive spectroscopy (EDS) is shown in figure 4. The EDS spectra confirm the presence of prominent Titanium [Ti] and oxygen [O] in the sample T5. From the EDS pattern, it is confirmed that, TiO_2 micro flowers synthesized by hydrothermal method belong to TiO_2 (1: 2). The amount in percentage and existence of each element in the sample is determined by scanning the sample at different sites. The average weight and atomic percentage of titanium in T_5 sample are 64.83 % and 38.11% and for oxygen are 35.17 % and 61.89 %, respectively as shown in table 1.

Table 1. The average weight and atomic percentage of TiO_2 (T_5) sample

Element	Weight %	Atomic %
Ti (L)	**64.83**	**38.11**
O (K)	**35.17**	**61.89**

IV. Conclusions:

The rutile TiO_2 micro flowersconsisting of nanorods onto a FTO substrate for boosting the photo electrochemical performance are successfully synthesized by simple surfactant free hydrothermal method. The SEM study reveals that micro flowers are made from numerous nanorods having average length of 3.70 μm growing homo centrally, display open structure, extended outside and becomes gradually compact inside. The crystallite size of the sample is found to be 41.9 nm.

V. Acknowledgment:

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TOXICICOLOGICAL STUDY OF CYPERMETHRIN AND ITS METABOLITES ON EARTHWORM (*EISENIA FETIDA*)

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ABSTRACT: *Cypermethrin, a synthetic pyrethroid is used in agricultural to control pests on variety of crops. It is a broad spectrum insecticide that acts mainly on the nervous system of vertebrates and invertebrates. Earthworms are common soil organisms that play an important role in improving texture, structure and soil aggregation, physical and chemical properties of the soil with improved fertility. However, they are sensitive and susceptible to agrochemicals because they lack hard cuticle around their body. The present paper deals with the toxicological studies of cypermethrin and its metabolites 2-propionic acid, benzoic acid and chlorine that were produced by a newly isolated strain of Paracoccus siganidrum APGM1. The toxicity was assessed by 48 hrs filter paper contact test, 14 days soil test and histopathological methods. The results of filter paper contact test revealed that the cypermethrin and its degraded metabolites vary in their contact toxicities. Earthworms were more susceptible to cypermethrin than their metabolites. The LC_{50} value of cypermethrin was 6 ppm while LC_{50} value of metabolites was 20 ppm. Fourteen days soil test are showed that at 6 ppm concentration of cypermethrin, half numbers of earthworms were died after 14 days and with the increase in the concentration, mortality was increasing. At 10 ppm concentration, all earthworms were died. However, half numbers of earthworms were died after 14 days at the metabolites concentration of 20 ppm. The histopathological results also indicated the adverse effects of cypermethrin and very little effect of metabolites on the morphological properties and structural integrity of the tissues. Thus, it was concluded that the metabolites of cypermethrin are less toxic to earthworm than the cypermethrin.*

Keywords: *Cypermethrin, metabolites, histopathology, filter paper contact test, 14 days soil test.*

I. INTRODUCTION

Synthetic pyrethroids occur constantly in some environments due to their wide use and repeated application. Due to their extremely hydrophobic properties, pyrethroids toughly bind to soil constituents and organic material. This permits pesticides to leach into the ground water and to form residues. This in turn is harmful to the ecosystem. Usually cypermethrin exists as a combination of *cis* and *trans* isomers (Kidd and James, 1991). The *cis* isomers are more active than *trans* isomers. There is no much more difference between the photo degradation rates of these two isomers in soil (Takahashi *et al*., 1985).

Earthworms are common soil organisms that play a major role in increasing texture, structure and soil aggregation, physical and chemical properties of the soil (Wang *et al.,* 2012). They represent up to 60 to 80% of the total animal biomass in soil (Olette *etal.,* 2008; Jouquet *et al.,* 2010). Earthworms are sensitive and susceptible to soil chemicals especially agrochemicals because they lack hard cuticle around their body (Lanno *et al.,* 2004; Nahmani *et al.,* 2007). Pyrethroids cause significant reduction in earthworm populations (Lukowicz - Ratajczak J. and Krechniak J., 1992). Earthworms are also highly susceptible to changes of ecological influences, mostly those intrinsic to the soil and earthworm behavior can therefore imitate soil contamination. It is known that earthworms reflect changes taking place in the soil, particularly changes of soil physical, chemical and biological properties, as well as changes in the water, air and thermal systems (Suthar *et al.,* 2008). Globally earthworms are used as bioindicators for investigating chemical environmental pollution.

There is a vital advantage of using microorganisms for degradation of pesticides. This is due to their diversity, wide dispersal and adaptation of variable metabolic pathways. The gene clusters are involved in microbial degradation. Many bacteria have potential to degrade the pyrethroid pesticides including cypermethrin. In the present study, cypermethrin and its metabolites such as 2-propionic acid, benzoic acid and chlorine produced by a newly isolated strain APGM1 of bacteria *Paracoccus siganidrum* was used to determine the toxicity of cypermethrin and its metabolites to earthworm.

II. MATERIAL AND METHODS

Earthworms:

Earthworms (*Eisenia fetida*) used in the experimental work were obtained from the earthworm culture farm in Kolhapur, India. Healthy adult worms with a well developed clitellum (average weight, 200-

250 mg) were used for study.

48 hours Contact Filter Paper Test:

Different concentrations of cypermethrin pesticide (1 to 10 ppm) were prepared in distilled water. Similarly, different concentrations of metabolites (1 to 10 ppm and above) from ethyl acetate extract of degradation medium were prepared. Filter paper pieces were treated with the different concentrations of the pesticides as well as metabolites and placed in a petridish. Earthworms were placed on the top of filter papers. The set up was replicated three times for each of the concentration and a similar design was set up using distilled water as a control. The dishes were incubated in the dark at 20 ± 1^0C for 48 hours and mortality was recorded at 12 hrs of time intervals.

14 Days Soil Test:

The natural soil was obtained from the local field, it was homogenized, air dried and sieved through 2 mm mesh. It was mixed with different concentration of pesticide (1 to 10 ppm) and its metabolites. 600 g of pesticide mixed soil was taken in the plastic container of 3.5 liter capacity and 10 earthworms were placed on each container and allowed to borrow. The earthworms were fed with urine free dried and grinded cattle manure (cow dung's) throughout the period of the experiment. This set up was repeated in three replicate for all the concentrations and control were also prepared using distilled water. Mortality of earthworms was evaluated on daily basis to determine the LC_{50} of the pesticide. To check the mortality, the test containers were emptied on a clean tray and earthworms were separated from the soil. Earthworms were judged to be dead when they fail to respond to gentle mechanical stimulations with a blunt probe.

Statistical Analysis:

Probit analysis had been used to determine the LC_{50} value at 95 % Confidence level using SPSS.

Histopathalogical study of earthworm:

Live earthworms from each treatment including the control, metabolites of pesticide and pesticide were taken and washed with distilled water. They were transferred into jars containing agar gel and left for another 96 hours to facilitate the removal of the sand content of the intestine, as agar is easily eaten by earthworm (Pokarzheyskii *et al*., 2000; Gobi *et al*., 2004).

By using routine paraffin method the histology of intestine of earthworm was performed (Humason, 1979). Intestine of earthworm, dissected out from the control and experimental animals, were blotted free of mucus, washed thoroughly in physiological saline, cut into pieces of desired size and fixed in Bovines fluid fixative immediately after autopsy. Fixation was carried out at room temperature for 24h, after which the tissues were transferred to 70% alcohol. Several changes of 70% alcohol were given until the yellow colour disappeared from the tissues. The tissues were then dehydrated by passing through ascending grades of alcohol, cleared in xylene, infiltrated with molten paraffin, and finally embedded in paraffin wax (58^0C MP).

Tissue sections of 5-µm thick transverse sections were obtained using a rotary microtome (Leica, Germany). The sections obtained were stained in Harris hematoxylene and eosin, dehydrated using alcohol, cleared in xylene and mounted using dihydroxy phthalate xylol (DPX). The stained slides were observed in a CarlZeiss (Germany) Axio-2 Plus research microscope.

III. RESULTS AND DISCUSSION

48 hours Filter Paper Contact Test (LC_{50} determination):

The results of filter paper contact test are presented in Tables 1. It shows that the cypermethrin and its degraded metabolites varied in their contact toxicities. Earthworms were more susceptible to cypermethrin than their metabolites. The LC_{50} value of cypermethrin was 6 ppm while LC_{50} value of metabolites was 20 ppm. Hence metabolites of cypermethrin degradation are less toxic to earthworm than cypermethrin.

Table 1: LC_{50} Determination

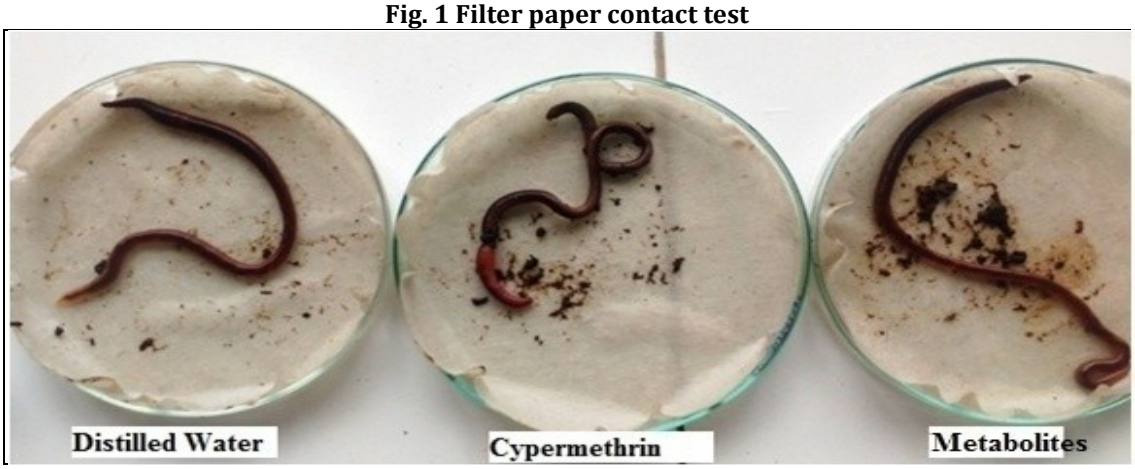
Concentration of Cypermethrin (ppm)	No. of exposed Earthworm	Mortality	Concentration of metabolite (ppm)	No. of exposed Earthworm	Mortality
1	10	1	1	10	0
2	10	2	2	10	0
3	10	2	3	10	0
4	10	3	4	10	0
5	10	4	5	10	0

6	10	5	6	10	0
7	10	6	7	10	0
8	10	8	8	10	1
9	10	9	9	10	2
10	10	10	10	10	3
-	-	-	20	10	5
-	-	-	30	10	8
-	-	-	40	10	10
Control Distilled Water	10	0	**Control D/W**	10	0

For cypermethrin
Spearman Karber Trim : 10.00%
LC50 : 6 pmm
95% Lower confidence : 0.02
95 % Upper confidence : 0.03

For Metabolites
Spearman Karber Trim : 10.00%
LC50 : 20 ppm
95% Lower confidence : 0.03
95 % Upper confidence : 0.04

Fig. 1 Filter paper contact test



Fourteen days soil test (LC_{50} determination):

The results of soil test are as presented in Table 2. It shows that at 6 ppm concentration of cypermethrin, half numbers of earthworms were died after 14 days and with the increase in the concentration, mortality is increasing. At 10 ppm concentration, all earthworms were died.

The metabolites of degraded cypermethrin were also used to check its toxicity on earthworm where it is found that at concentration of 20 ppm of metabolites (LC_{50} value of 20) half numbers of earthworms were died after 14 days.

Histopathological examination of intestinal sections of control earthworm showed normal structure of wall and intact nature of circular and longitudinal muscles (Fig. 2C). In the earthworms exposed to cypermethrin, disintegration of ectodermal layer as well as circular and longitudinal muscles was found. It may be due to the necrotic effect of cypermethrin pesticide at LC50 (6ppm) for 48 h. There was a total damage of body wall of the *Eisenia fetida* due to the internal and ectodermal tissue erosion (Fig. 2P). However, earthworms exposed to cypermethrin metabolite at LC50 (20ppm) for 48 h, revealed normal structure of body wall and expansion of spaces between the longitudinal muscles with proliferation of glandular cells (Fig. 2M). These results indicate the adverse toxic effects of cypermethrin and very little effect of metabolites of cypermethrin on the morphological properties and structural integrity of the tissues.

Contact filter paper test is reported to be an excellent screening technique to assess the relative toxicity pesticides to the earthworms. It is one of the simpler, cheaper and quick method. It is designed in such a way that the earthworms are exposed to the toxicant both by contact and in the aquatic phase. (Edwards and Bohlen, 1996). According to the regulation of environmental risk assessment for agricultural pesticides, the suggested standard of toxicity are LC_{50}< 1 mg kg^{-1} for highly-toxic pesticides, 1–10 mg kg^{-1} for medium-toxic pesticides, and > 10 mg $kg^{-1}$for low-toxic pesticides (MEPPRC, 1990). With this standard, the acute toxicity of cypermethrin on earthworm was found to be medium, while acute toxicity of degraded metabolites was found to be low. Yuguda *et al*. (2015) also assessed the toxicity of some pesticides on

earthworms (*lumbricus terrestris*) using 48 hours contact filter paper test and 14 days soil test. They found that all the pesticides were toxic to earthworms based on LC_{50} values of 48 hours contact filter paper test while difference in toxicity profile in fourteen days soil test that was based on the pesticides classes. Soil test is a more representation of natural environment of earthworms and the pesticides are mainly absorbed by gut in this method (De Silva and Van Gestel, 2009, Udovic and Lestan, 2010). Therefore the soil test is more adequate when toxicity of pesticides to earthworms is to be evaluated (Wang *et al.,* 2011).

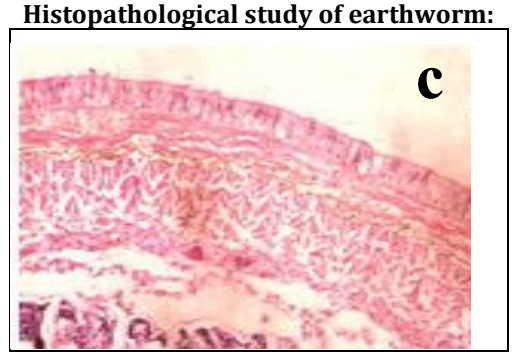
Table 2: LC_{50} Determination

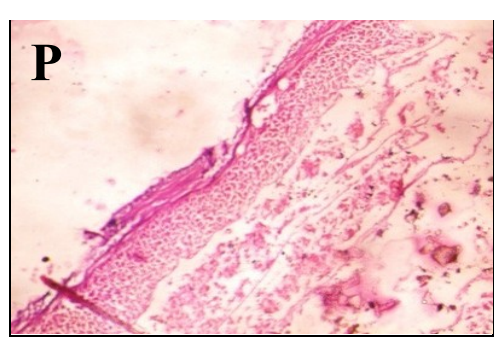
Concentration of Cypermethrin in soil ($mgkg^{-1}$)	No. of exposed earthworms	Mortality after 14th days	Concentration of metabolites ($mgkg^{-1}$)	No. of exposed earthworm	Mortality after 14 th days
1	10	1	1	10	0
2	10	2	2	10	0
3	10	2	3	10	0
4	10	3	4	10	0
5	10	4	5	10	0
6	10	5	6	10	0
7	10	6	7	10	0
8	10	7	8	10	1
9	10	8	9	10	1
10	10	10	10	10	3
			20	10	5
			30	10	7
			40	10	10
Control	**10**	**0**	**Control**	**10**	**0**

For cypermethrin
Spearman Karber Trim : 10.00%
LC50 : 6 $mgkg^{-1}$
95% Lower confidence : 0.02 95%
95 % Upper confidence: 0.03 95 %

For Metabolites
Spearman Karber Trim: 10.00%
LC50 : 20$mgkg^{-1}$
Lower confidence : 0.03
Upper confidence : 0.04

Histopathological study of earthworm:





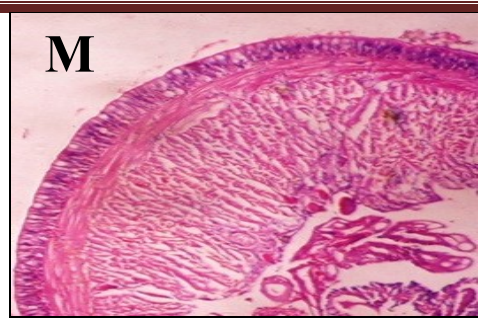


Fig. 2 C, P and M: Histology of earthworm intestine (C-control, P- Effect of cyeprmethrin pesticide, M-Effect of cypermethrin metabolites)

The toxicity of cypermethrin to the earthworms has also been measured previously by several workers. Inglesfield C. (1984) determined a 14-d LC50 of greater than 100 mg kg^{-1} and an acute (14-d) no observable effect of concentration of 100 mg kg^{-1} for alpha cypermethrin in artificial soil. The LC50 value of cypermethrin for *Eisenia fetida* was 26.11 Jg cm^{-2} (Roberts & Dorough, 1984).

Many previous reports on earthworms also suggest the morphological and histopathological changes on exposure of different toxic metals and organophosphate pesticides. Disintegrations of cuticular and ectodermal membranes were recorded by Amaral *et al.,* 2006. Landrum *et al*., (2006) discussed the survival rate of *Eisenia fetida* when exposed to Perchlorate in the form of disintegration of the peritoneum (chloragogenous layer) and the epithelium.

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ISOLATION, CHARACTERIZATION OF CAROTENOIDS FROM CRUSTACEAN (PRAWN) SHELL WASTE AND STUDY IT'S PHARMACOLOGICAL USES

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ABSTRACT: *Prawns are one of the most eaten sea food for its taste and nutrition. But its waste (shells) creates fearsome smell in garbage bins. Marine natural products have attracted biologists and chemists the world over. So it gained a commercial value as an industrial raw material. There is a growing interest in the functionality of natural colorants and pigments (Sang et al., 2002). Carotenoids are the most important among the pigments that are found in nature.*

The present study aims about isolation of carotenoids from prawn shell waste using different solvents and estimate the maximum yield. The isolated carotenoids were characterized by TLC method and compared with the Retardation Factor (Rf) as indicated by Lorenz Todd standard chromatogram. The extracted carotenoid content was determined spectrophotometrically at 470 nm. The antibacterial activity of extracted carotenoid sample was studied against micro-organisms.

The detection of pharmacological effects of carotenoids as Anti-fungal, Anti-oxidant, Anti-diabetic, Protection from UV rays was carried out.

Keywords: *Prawn shells,Carotenoids, TLC, Anti-bacterial, Anti-oxidant, Anti-diabetic, Protection from UV rays.*

I. INTRODUCTION

The ocean is earth's one of the most valuable natural resources. The sea is full of riches of living and non living resources. It provides food in the form of fish and shellfish, about 200 billion pounds are caught each year. The ocean is an increasingly important source of bioactive substances with enormous potential for fighting disease. The low yields of these compounds have proven to be a severe obstacle for the development of promising compounds. Initial identification of bioactives from marine sources paved the way for utilization of huge amounts of fish processing waste.

The crustaceans are one of the most used sea food which include shrimps, prawns and crabs etc. Only 40% part of it is used as food and remaining 60% is discarded as prawn shell waste which includes head portion and the shell. In addition to protein, lipid, $CaCO_3$and chitin, prawn shell is a good source of carotenoids which include β-carotene, lycopene, lutein, echinenone, mono and di-esters of astaxanthin, etc. Carotenoids are most important and numerous pigments found in nature. These factors produce yellow-red color in the plant and animal products.

These carotenoids occur in red coloured aquatic organisms e.g. Shrimps, Crabs, Prawns, Lobsters, and Salmon. These turn red when cooked because astaxanthin bound to protein in shell becomes free as protein denatures and unwinds and produces red colour.

Astaxanthin is a very potent antioxidant suitable for use as a drug or food supplement (FDA approved) in the treatment of cardiovascular, immune, neurodegenerative diseases and also cancer. Vitamin A is an essential micronutrient for animals essential for vision, growth, reproduction and normal development of skin and mucosa which is produced in animal body from β-carotene. Carotenoids act as antioxidants protecting cells and tissues from damaging effects of free radicals and singlet oxygen.

This present study is aimed at considering more useful purposes for this rotting waste by extracting the carotenoids. The residue available after extraction of carotenoids can be used for production of chitin and chitosan thus having an integrated approach for efficient utilization of prawn shell waste.

II. MATERIALS AND METHODOLOGY:

Collection of crustacean waste:

The crustacean (prawn) shell waste was collected from the fish market at Mangalwar Bazaar in Solapur, Maharashtra. The sample was collected in a polythene bag and transported to laboratory.

Raw material preparation:

The samples were washed under running tap water and dried at 50-60^{0}C in hot air oven for 20-24 hrs. The dry samples were homogenized in a laboratory mixer and sieved prior to extraction and estimation

of carotenoid pigments.

Extraction of Carotenoid Pigments:

Extraction of carotenoid pigments from crustacean shells was carried out using two different solvents- Acetone and Ethanol. 1 g of homoginised dry sample was extracted in 10 ml of either solvent for 1 hr on magnetic stirrer. Then the carotenoid extract was filtered using Whatmann No. 1 filter paper. Recovered crustacean shell waste was repeatedly extracted with fresh solvent until the filtrate was colorless. The extracts were pooled together and collected for future studies.

In case of acetone extraction the pooled extract was collected in a separating funnel, 12.5 ml of petroleum ether and 9.4 ml of 0.73% (w/v) NaCl solution were added. After through mixing the epiphase was collected. To the lower phase equal volume of water was added, mixed and the epiphase was collected, the pooled epiphase was evaporated to dryness.

Primary screening for phytochemical constituents:

The screening for phytochemical constituents in the extracts was performed using generally accepted laboratory technique for qualitative determinants.

Determination of carotenoids content:

The absorbance of each extract was measured using spectrophotometer at 470nm. The "blank" reference corresponded to the corresponding solvent used in extraction (Schiedt and Liaaen-Jensen, 1995). The concentration of carotenes in the extract was determined using the following equation:

$$\textbf{Carotenoids (mg)} = \frac{A \times \text{volume} \times 1000}{A^{1\%}_{1cm} \times 100}$$

$A^{1\%}_{1cm}$ for ethanol = 2500; $A^{1\%}_{1cm}$ for acetone = 2100

Thin layer chromatography to determine the different compounds:

The extract was analyzed by thin layer chromatography. On a TLC plate a line 1cm above from bottom was drawn and spot the extract on the line three times. Allow the spot to dry. Keep the plate in chromatographic chamber which contain solvent mixture as Benzene: Ethyl acetate (1:1). The separated bands were identified using internationally accepted Rf values for astaxanthin monoester and astaxanthin diester given by Lorenz Todd, 1998.

Anti-bacterial activity:

Agar diffusion assay is used widely to determine the anti-bacterial activity of Leaf extracts. Nutrient agar prepared was poured in the Petri dish. 24 hours growing culture were swabbed on it. The wells were made into the plates for adding the control and sample. Inhibition zones were evaluated after incubating the plates at 37^0C for 24 hrs.

The Gram positive organisms-Staphylococcus aureus, Bacillus subtilis and Gram negative organisms- Salmonella typhi, Pseudomonas aeruginosa were used for determination. Chloramphenicol was used as standard.

Test for determining antioxidant activity - Test of DPPH

The sequestration of the DPPH radical was determined method by this method. 3ml of methanolic solution of 0.15mM DPPH and 1ml of the carotenoid extract were mixed. The "blank" corresponds to the mixture of 3ml of methanol and 1ml of carotenoid extract. The "control" corresponded to the methanolic solution of DPPH. The absorbance of the mixtures was measured at 517 nm after incubation in dark for 30 minutes using UV-Vis Spectrophotometer. The scavenging activity was calculated using the following equation:

% Scavenging = [(Absorbance of control - Absorbance of test sample)/Absorbance of control] X 100

Test for determining protection from UV-rays

The UV absorption spectrum for the extract was obtained in the range of 200–400 nm using UV–visible spectrophotometer to determine absorbance of the extracts in the UV range.

Test for determination of anti-diabetic activity - α- amylase inhibition assay:

α- amylase (diastase) from fungal source was used. The enzyme was diluted using 0.1M phosphate buffer (pH6.9).The α-amylase inhibitory activity was determined according to a modified procedure described by Zia-Ul-Haq et al. (2011). Briefly, 20µl of the enzyme solution (1Uml-1) was pre-incubated with20µl of extract or purified pigment, at 37^0C for10min. After the incubation, 250µl of 1% starch in 0.1M phosphate buffer (pH6.9) was added and the mixture was further incubated at 37^0C for 20min. The reaction was terminated by adding 200µl of 3, 5-dinitrosalicylic acid (DNSA reagent). The tubes were incubated in a boiling water bath for a few minutes. 5mL distilled water was added to all the tubes and their absorbance was recorded at 540nm against a blank devoid of starch and sample. The control reaction, signifying 100%

enzyme activity contained buffer or DMSO instead of the samples. Acarbose was used as a positive control. The inhibitory activity was calculated by using the following equation:

% inhibition = ($A_{control} - A_{sample}$) / $A_{control}$ x 100

III. RESULT AND DISCUSSION:

Extraction of Carotenoids from crustacean shell wastes:

Extraction of carotenoid pigments from the crustacean shell wastes were performed using two different organic solvents. The solvent extracted carotenoid was in the form of orange-red coloured solution.

Screening of phytochemical constituents:

Investigations on phytochemical screening of compounds in the extracts showed the presence of terpenoids. Carotenoids belong to the group of terpenoids.

Determination of carotenoid content: The carotenoid content was obtained to be 0.0074mg for ethanolic extract and 0.0177mg for acetonic extract. The content of carotenoid obtained was much less than that stated by G. M. Lira et al. who reported it to be 0.5-3.5 mg.

Identification of different carotenoids by TLC:

Identification of pigments was investigated by TLC where three bands were obtained and Rf values of three bands were compared with the standard values stated by Lorenz Todd, 1998. The acetonic extract gave more separated and prominent bands than the ethanolic extract

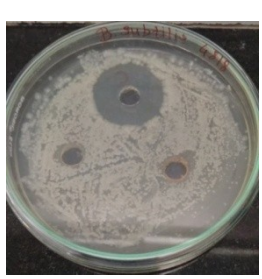
Carotenoid	Typical Rf value
β-carotene	0.99
Echinenone	0.87
Astaxanthin Di-esters	0.75
Astaxanthin Monoesters	0.50
Canthaxanthin	0.40
Astaxanthin free	0.33
Lutein	0.25

The obtained Rf values were 0.95, 0.89, 0.74 which vary slightly from the standard stated values and correspond to β-carotene, echinenone and astaxanthin di-esters. The Rf values obtained for astaxanthin diester are in agreement with the results reported by Kobayashi and Sakamoto which had Rf values indicated as 0.75-0.85 for astaxanthin diester

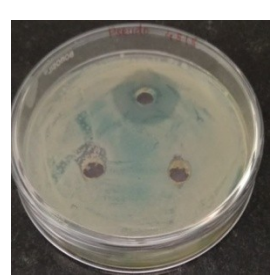
Anti-bacterial activity:

Agar well diffusion assay was used for study of antibacterial activity. The results obtained are less than that stated in two reference works of U.N Ushakumari and R. Ramanujan(2013), Dalei and Sahoo(2014). These results are due to less carotenoid content in extract than that mentioned in the above works.

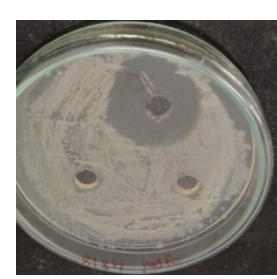
Bacterial Strains	Zone of inhibition in mm		
	Control	Acetonic extract	Ethanolic extract
Pseudomonas aeruginosa	20	6	5
Staphylococcus aureus	13	3	2
Salmonella typhi	22	4	5
Bacillus subtilis	21	2	3



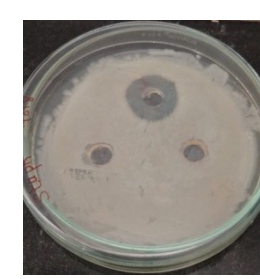
Bacillus subtilis



Staphylococcus aureus



Salmonella typhi



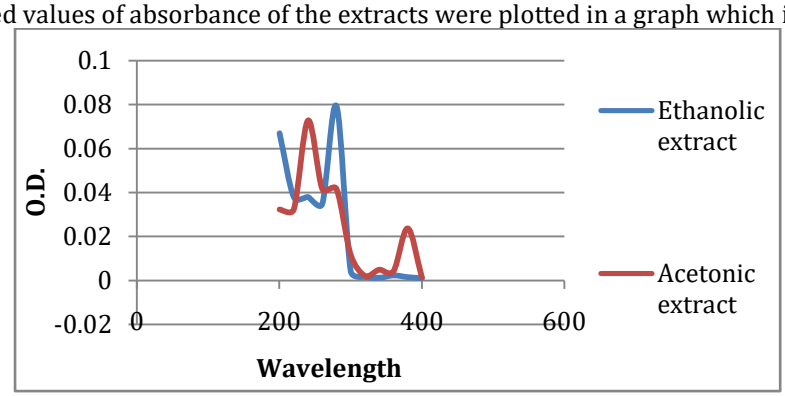
Pseudomonas aeruginosa

Antioxidant activity:

The ability of the studied extracts as scavangers of DPPH•, expressed as a percentage of the antioxidant activity. The total antioxidant capacity (TAC) for acetonic extract is 78.06% while that of ethanolic extract is 99.58%. The obtained values are higher than that mentioned by G.M. Lira et al.

Protection from UV rays:

The obtained values of absorbance of the extracts were plotted in a graph which is as follows:



The results obtained showed the extract has ability to absorb UV radiation, hence it's proved UV protection. However, the ranges giving maximum absorption were in UV B wavelength range.

Anti-diabetic activity:

The α-amylase inhibition assay was used to investigate the anti-diabetic activity of the extracts. The positive control acarbose showed the inhibition activity 66%. The acetonic extract of carotenoids showed exactly similar results as that of positive control i.e. 66% amylase inhibitory percentage. The ethanolic extract of carotenoids had 50% inhibitory activity towards action of amylase on starch.

IV. CONCLUSION

Crustacean shell wastes from industries and markets can be utilized for the isolation of important bioactive compounds like natural carotenoids as stated in this work. The different pharmacological activities stated can make the shell waste to become a potent raw material for pharmaceutical industries for production of respective activity drugs. This will also reduce the fearsome smell caused due to the rotting waste of prawn shells in surrounding environment of their dumping sites.

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SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING PLANT ROOT EXTRACT AGAINST URINARY TRACT INFECTION PATHOGEN

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ABSTRACT: *Nanotechnology becomes a buzz word now all over the world. Nanotechnology is a multidisciplinary subject. Zinc oxide nanoparticles show its antimicrobial activity against Urinary tract infecting pathogens viz.E.coli, klebsiella sp., Pseudomonas sp., Staphylococcus sp. Nanoparticles were synthesized using Hemidesmus indicus root extract. Nanoparticles were characterized by UV-visible spectroscopy, Fourier transform infrared spectroscopy. The use of plant in nanoparticle synthesis is novel leading to green chemistry. Zinc oxide nanoparticles using Hemidesmus indicus root extract have been tested against Urinary tract infecting pathogens. This study indicates the synthesis of nanoparticles using biological method is less investigated method.*

Keywords: *Nanotechnology, Zinc oxide nanoparticles, Hemidesmus indicus, Urinary tract infecting pathogens.*

I. INTRODUCTION

Nanotechnology involves the use of materials having nanoscale dimensions in the range of 1-100nm. Operating with nanomaterials has allowed researchers to have a much better understanding of biology. The green synthesis of nanoparticles has greatly reduced the use of physical and chemicals methods. Various chemicals methods have been proposed for the synthesis of zinc oxide nanoparticles (ZnO NPs), such as reaction of zinc with alcohol, vapor transport, hydrothermal synthesis, precipitation method etc. The use of green synthesis method by the researchers is rapidly increasing due to usage of less toxic chemicals, eco- friendly nature and one step synthesis of nanoparticles [1].

Zinc Oxide is a mineral that acts as skin protecting. The inorganic antimicrobial agent, such as metal oxides, has received increasing attention in food applications because they not only stable under high temperature and pressure but they are also generally regards as safe for human beings and animals relative to organic substances. Zinc Oxide is listed as '' generally recognized as safe" [GRAS] by the US food and drug administration [2]. Nano sized particles of ZnO have more pronounced antimicrobial activates than large particles since the small size [less than 100nm] and high surface to volume ratio of nanoparticles allow for better interaction with bacteria. ZnO NP have been shown to have a wide range of antibacterial activities against both Gram-positive and Gram- negative bacteria, including major pathogen like *E. coli*, *Salmonella. Listeria monocytogenes,* and *Staphylococcus aureus* [2]. However, a few studies have suggested that the primary cause of antibacterial function might be from the disruption of cell membrane activity [2]. Among those, ZnO NPs are used in the elimination of toxic chemicals like arsenic, sulfur from water source owing to their large surface area by volume ratio than the bulk materials diagnostics, optoelectronic devices, biomolecular detection, surface acoustic wave devices like laser devices, electromagnetic coupled sensor. They can act as an alternative source for degradation of atmospheric pollutants [1].

Hemidesmusindicus is a widely used shrub in Indian folk medicine and considered as magical spiritual dream herb in Ayurvedic medication. It is used as vital herb for healing many aliments and to treat diversified diseases. This plant is found throughout India growing under mesophytic to semi dry conditions in the plains. It is quite common in open scrub jungles, hedges uncultivated soil. It is found in India, Sri Lanka, Pakistan, Iran Bangladesh and Moluccas [3]. It is a well known traditional medicinal plant widely used in ayurveda, Siddha and Unani systems of medicine to treat a variety of diseases such as chronic fever and asthma, urinary tract infection blood purifier. The use of herbal drugs is increasing worldwide as they have fewer or no side effect as compared with synthetic drugs [3]. This plant is a good source of different bioactive chemical compound like Hemidesmin-1 and Hemidesmin-2, α-amyrin, β- amyrin, lupeol acetate, β-sitosterol, hemidesmol and hemidesterol which were responsible for many of the pharmacological activates [3].

Synthesis of nanoparticles using biological method involves use of biomembranes, DNA, enzymes and microorganisms. Many of the materials sysnthesized by microorganisms, animals and plants in nature can indeed be synthesized using them in laboratories even on large scale. This is considered to be a very attractive possibility so as to have eco-friendly or so-called green synthesis. Use of plants in synthesis of

nanoparticles is quite novel leading to truly green chemistry that technologist are looking for. However, compared to use of microorganism to produce nanoparticles, use of plant extracts is relatively less investigated [4]. It was reported that zinc oxide nanoparticles has showed high antibacterial activity against urinary infection disease. Further the antibacterial activity of these biological synthesized zinc oxide nanoparticles was evaluated against different pathogenic microorganisms [1].

II. MATERIALS AND METHODS

1] Preparation of plant extract:

5 gm of dry root (Fig.1) were washed with running tap water followed by double distilled water and then soaked in 200 ml conical flask containing 100 ml distilled water (Fig.2).The solution was boiled at 70° C for 10 min. the root extract was allowed to cool to room temperature filtered through whatman number 1 filter paper, and the filtrate was stored for further experimental use.

Fig.1 – Dry roots of *Hemidesmus indicus*.

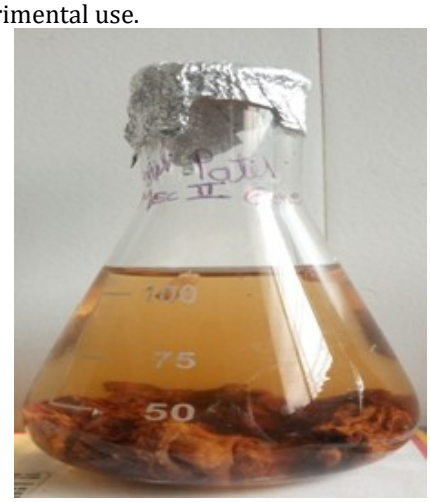


Fig.2 - soaked roots in conical flask

2] Synthesis of ZnO nanoparticle:

1 mM zinc acetate [Zn $(O_2CCH_3)_2(H2O)_2$] was dissolved in 50 ml double distilled water and kept in stirrer for 1 hr respectively. Then 20 ml of NaOH solution was slowly added into zinc acetate solution and 25 ml of plant extract was added to the same. The color of reaction mixture was changed after 1 hr of incubation time. The solution was left in stirrer for 3 hr yellow color appeared. After the incubation time confirmed the synthesis of ZnO nanoparticle. The precipitate was separated from reaction solution by centrifugation at 10000 rmp at 60°C for 15 min and supernatant was collected. The supernatant were cool at room temperature. The solution was kept in oven for 2hr at 90°C. The powder was formed. Preserved the powder in air-tight bottles for further studies.

3] Characterization of biosynthesized ZnO NP's

Optical properties of ZnO NP's were characterized based on UV absorption spectra with the wavelength range of 300-500 nm.

3.1] Sample preparation for FT-IR

Sample preparationis a difficulttask in IR range as there is no transparent material for cuvettes alkali halides [such as KBr and NaCl] are usually used which are transparent even at longer wavelengths. 1 mg of ZnO NP was mixed with 100 mg of potassium bromide (FTIR grade) and pressed into a pellet for FTIR characterization. The sample pellet was placed into the sample holder and FTIR spectra were recorded in FTIR spectroscopy at a resolution of 4 cm^{-1}.

4] Antimicrobial activity of synthesized ZnO NPs

Antimicrobial activity of synthesized ZnO NPs was performed by disc diffusion method against Urinary tract infecting pathogens viz.*E.coli, klebsiella sp., Pseudomonas sp., Staphylococcus sp.* Fresh overnight culture of each strain was spread uniformly onto the individual plates. Ampicillin disc were used as a control. The ZnO NPs solution impregnated disc were placed onto the plates and incubated for 24h at 37°C. After incubation period got over, different levels of zonation formed around the disc.

III. RESULT AND DISCUSSION

1. Visual observation

ZnO NP has attracted great attention because of their superior optical properties. Visual color change is the preliminary test for nanoparticle synthesis. Represent the synthesis of ZnO NP synthesized

using freshly prepared *H. indicus* root extract. Color change from brown to pale yellow represent the synthesis of ZnO NP.

Fig.3 - Visual observation of ZnO NP synthesis a] initial colorb] final color change

Fig.3.a

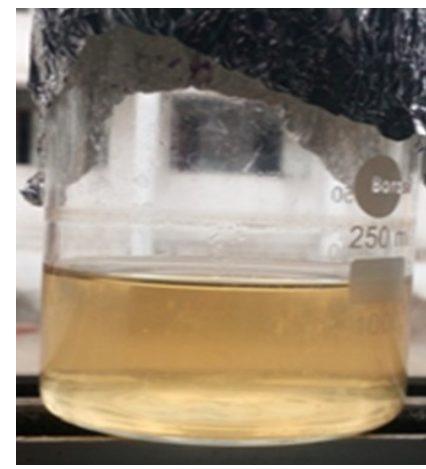
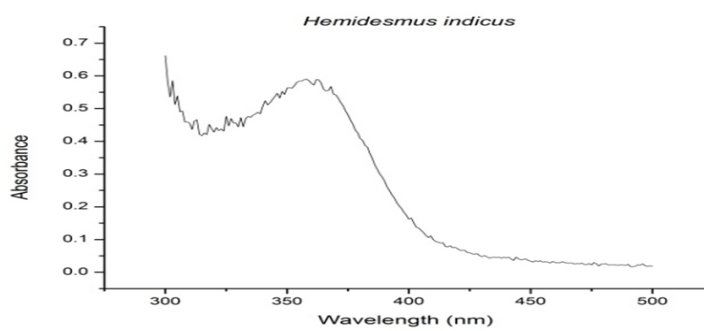


Fig.3.b

2. UV-visible analysis

UV-visible spectroscopy is usually conducted to confirm the synthesis of ZnO NP. Conducting electrons start oscillating at a certain wavelength range due to surface Plasmon resonance (SPR) effect. Represent the UV-visible spectra of freshly prepared ZnO NP. Peak obtained at 371 clearly demonstrates the presence of ZnO NP in the reaction mixture. (Fig.4)

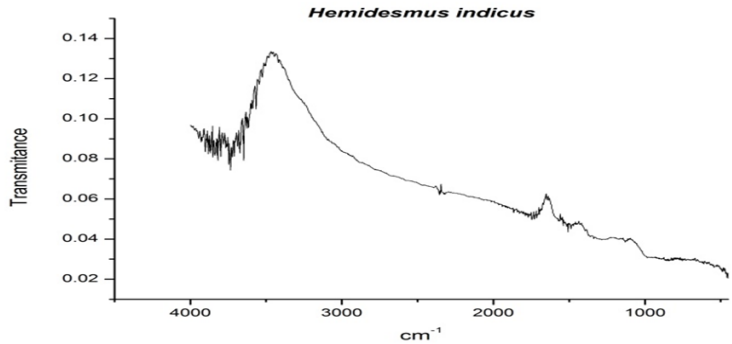
Fig.4- UV- vis spectrum of ZnO NP synthesized by *H. indicus.*



3. FT-IR analysis

Substance- specific vibrations of the molecules lead to the specific signals obtained by IR spectroscopy. FT-IR spectra and functional group involved in ZnO NP synthesis illustrated peak in the range of 500-4000 cm^{-1} broad peak obtained at 3457 corresponded to OH stretching bond vibrations because of water adsorption on the surface of zinc oxide nanoparticles (Fig.5)

Fig.5- FT-IR spectrum of ZnO NPs



4. Antimicrobial activity

Disc diffusion technique was adapted to perform the assay. Anti-bacterial effect of ZnO Np was visualized against urinary tract infection pathogen viz.*E.coli, klebsiella sp., Pseudomonas sp., Staphylococcus sp.*Results clearly demonstrate that the nanoparticles showed anti-bacterial effect. The zinc oxide nanoparticles are inhibit the microbial growth *in-vitro* antimicrobial activity.

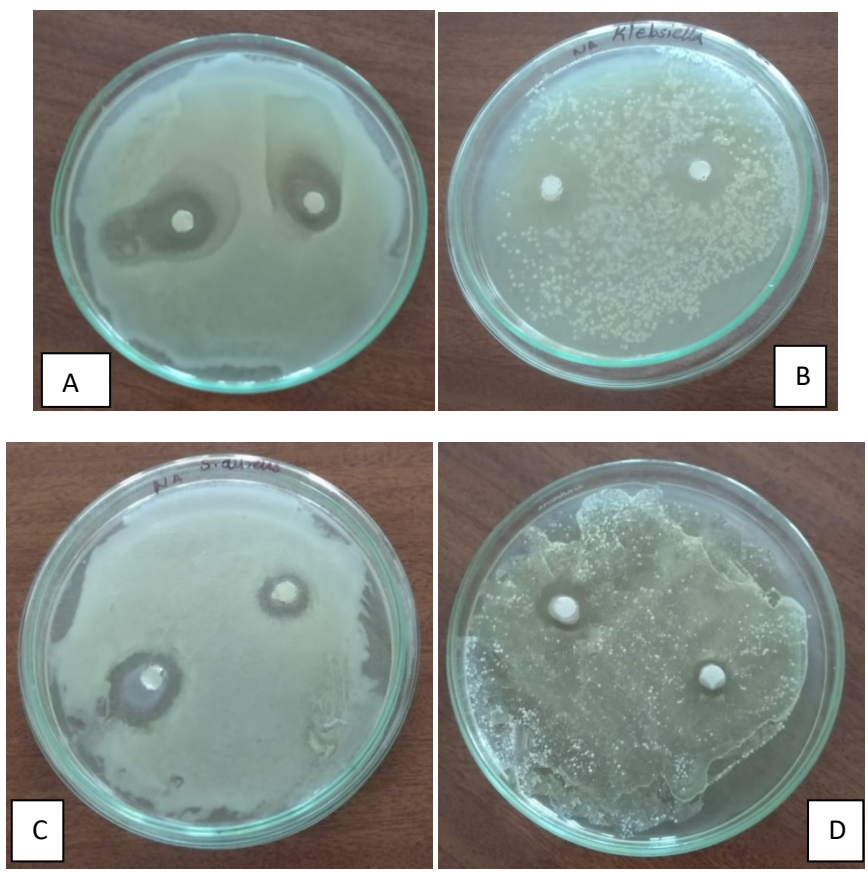


Fig.6- Antimicrobial activity against A] *E.coli B] klebsiella sp.* C] *Pseudomonas sp.* D]*Staphylococcusaureus*

IV. CONCLUSION

Green synthesis of zinc oxide nanoparticle using *Hemidesmus indicus* root extract gives successful path for eco- friendly method of synthesis of nanoparticles. The presence of various biologically active compounds found in the root extract helps in the synthesis of zinc oxide nanoparticles. As a preliminary confirmation, the rapid synthesis of ZnO NP was measured using the UV-visible spectroscopy at maximum absorbance at 371nm. ZnO NP showed its antimicrobial activity against Urinary tract infecting pathogens viz.*E.coli, klebsiella sp., Pseudomonas sp., Staphylococcus sp.*

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ANTIMICROBIAL ACTIVITY OF *PIMENTA DIOICA* AGAINST PATHOGENIC MICRORGANISMS

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ABSTRACT: *The genus Pimenta dioica very widely distributed throughout the world. Roots and exctract of Pimenta dioica have been used for the treatment of inflammation and other disorders but it may have some other potential medicinal properties. The aim of present study was to determine antimicrobial activity of Pimento dioica (native name Allspice). Acetone and water extracts of fresh leaves of Allspice plant at a final concentration 30 mg per ml were tested against micro-organisms S. aureus, E. coli, P. mirabilus, B. subtilis, P. aeroginosa using agar well diffusion method.The plant extract exhibited antimicrobial activity against all the test microorganisms. E. Coli, Pseudomonas aeruginosa was the most susceptible to the plant extract as compare to other microorganism. Pimento dioicacould be a potential source of antimicrobial agents.*

Keywords: *Antimicrobial activity, antimicrobial agents*

I. INTRODUCTION

The man and animal depend upon the plant our environment characterized by rich diversified plant life. The plant diversity composed of more than 5,00,000 botanical Spp.on a global basis atleast130 drugs. All single chemical entities extract from higher plant or modified further synthetically are currently in use (Sreenu Pendali, Samantha Talari, 2014)

Medicinal plants are grate values which were used in traditional system of medicines neutraceutical food supplements. Folk medicines modern medicines and pharmaceutical intermediate because plants are the richest bio resources of the drug (Sreenu Pendali, Samantha Talari,2014).

The use of tradition medicines a common practice in developed and developing countries for their primary health care level, the medicinal plant posse's chemical substance generally term as bioactive compound. The world health organization report reveals that 75% - 95% of developing countries the population depends upon traditional medicines and traditional therapies. It is need to look forward to research on herbal medicinal plant for validating the ethanol medicinal used and subsequent isolation and characterization of biological active compound. For making potential drug in developing countries because huge population live in poverty and suffering safe medicine. (Sreenu Pendali,2014). Medicinal plantrepresent rich source of antimicrobial agent. The different part used include roots stems flowers fruits twigs and modified plant although hundreds of plant species have been test for antimicrobial properties.

The *Pimenta dioica* is well known medicinal plant commonly known as allspice. it also called Jamaica pepper, pepper myrtle pepper pimenta Turkish yenibahar of new spice is a dried unripe fruit (baries used as spice) of *Pimenta dioica* a native of Antilles southern, Mexico and central America, cultivated in many warm, coins as early 1621 by English who throughout combined flavor cinnamon, nutmeg, cloves. The all spices tree classified as an evergreen shrub can reach 10 – 80 cm [33 – 59 ft.] in height. It can be grown outdoors in tropics and sub-tropics with normal garden soil it adapts well to container culture and can kept as house plant or in a greenhouse. (Priya S Rao,Sheth Navinchandra,2012)

Most of the work done on *Pimenta dioica* stem, roots, oils of leaves but their no satisfactory work on the leaves of *Pimenta dioica* so I am choosing leaf extract it also used for medicinal the measure group of chemical constituent presenting the *Pimenta dioica* such as carbohydrates, flavonoides, terpenoides, steroids. (O.TURGAY and Y.ESEN;2015)

Pimenta dioica show antimicrobial activity and antioxidant activity [Mukesh KR.singh et.al] Antimicrobial is an agent that kills microorganisms or stops their growth agent that kills microbes are called microcidal. While those merely inhibit their growth are called biostatic(Webster online Dictionary).

II. MATERIALS AND METHOD

Sample Collection

Fresh leaves of spices were collected from botanical garden of Yashvantarao Chavan Institute of Science, Satara. The allspices leaves were washed with the help of tap water Followed by distilled water. The

leaves were grinded with the help of electrical mixer.

Culture preparation

The test organism were selected from each of the following species namely *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeroginosa* available in department of microbiology Y.C. institute of science, Satara.

Table no. 1 Antimicrobial activity

Sr. No.	Organism used	Antimicrobial activity
1	***Staphylococcus aureus***	+
2	***Escherichia coli***	+
3	***Proteus mirabilis***	+
4	***Bacillus subtilis***	+
5	***Pseudomonas aeroginosa***	+

3) Preparation of plant extract:-

- Water extract-

10gm of all spice leaves were washed with tap water followed by distilled water. After repeating washing leaves were air dried and powder was prepared with the help of electrical mixer. 10gm of powder soaked in 30ml of sterile distilled water. Then the extract was filtered by using filter paper in clean beaker

- Organic solvent extract

All spices leaves were washed with tap water followed by distilled water. After repeated washing leaves were air dried and powder was prepared by using mixer. 10gm of dry powder was soaked in 30ml of acetone. Then the extract were filtered by using filter paper and filled air tight cap bottles and used it for further experiments.

- Water Extract

Antibacterial activity is carried out by well diffusion method. Sterile plates were prepared by using nutrient agar medium and spread with suspension of *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeroginosa* respectively. There after three wells of 8mm diameter were dug with the help of sterile borer. One plate was prepared for each organisms add 0.1ml for each verity of Allspice extract prepared in sterile distilled water used in the study and distilled water in one well as a control. Incubate plates at 37^0C for 24 hours. After incubation plates were observed for zone of inhibition and result was note down.

- Acetone Extract-

Antibacterial activity is carried out by well diffusion method. Sterile plates were prepared by using nutrient agar medium and spread with suspension of *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeroginosa* respectively. There after three wells of 8mm diameter were dug with the help of sterile borer. One plate was prepared for each organisms add 0.1ml for each verity of allspice extract prepared in acetone used in the study and Acetone in one well as control. Incubate plates at 37^0C for 24 hours.

Sr. No.	**Organism Used**	**Zone of Inhibition**	
		WE[0.1ml]	**AE[0.1ml]**
1	***Staphylococcus aureus***	**2.3**	**3.5**
2	***Proteus mirabilis***	**2.5**	**3.4**
3	***Escherichia coli***	**2.9**	**3.5**
4	***Bacillus subtilis***	**2.7**	**3.0**
5	***Pseudomonas aeroginosa***	**2.9**	**3.5**

After incubation plates were observed for zone of inhibition and result was note down.

III. RESULT AND DISCUSSION

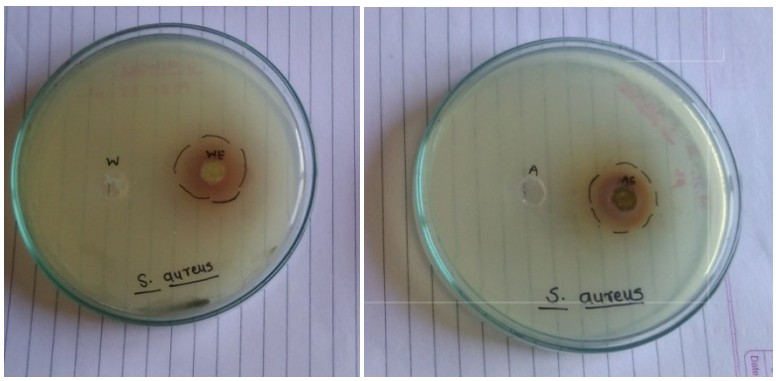

Fig.1 Water extract and Acetone Extract of Allspice showing Antimicrobial Activity against *S.aureus*.

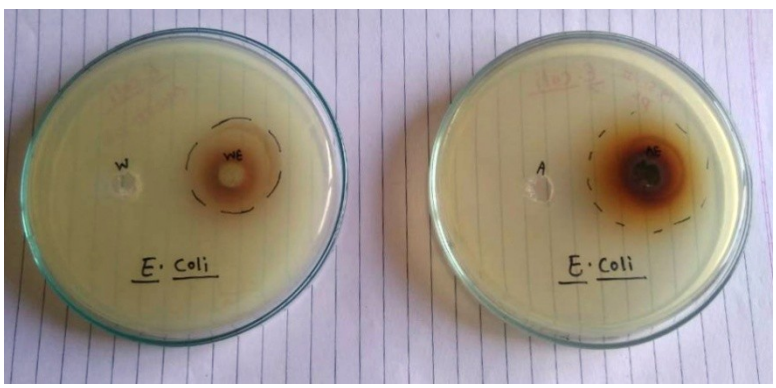

Fig.3 Water extract and Acetone Extract of Allspice showing Antimicrobial Activity against *E. coli*.

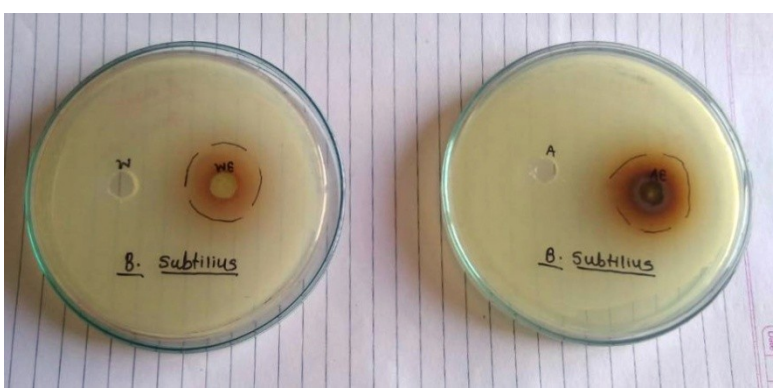

Fig.4 Water extract and Acetone Extract of Allspice showing Antimicrobial Activity against *B. substilis*

IV. DISCUSSION:

The result obtained showed that full concentration of acetone extract of pimenta dioica had inhibitory effect on one of the five tested microorganism as represented in table show that the all microorganism show inhibitory effect in high concentration the 0.1ml acetone extract and water extract.

The acetone extract at *Pimenta dioica* inhibitory effect on all microorganism the mean zone of inhibition found to be *E.coli* AE (0.1)3.5cm, S.aureus-3.5,P.aeroginosa 3.5cm,B.subtilis-3cm,in the mean zone of inhibition found to be E.coli (0.1)-2.9cm S.aureus-2.3cm,P.aeroginosa-2.9.

The *E.coli, P.aeroginosa* was the most susceptible to the plant extract as compare to other test organism.

V. CONCLUSION:

The present work demonstrates the antimicrobial potential of *Pimento dioica* leaves extract by

using various solvents. The result indicates that acetone extract are better than water for the extraction of the antibacterial properties of *Pimenta dioica*. The observed inhibition of bacteria, *E.coli, P.mirabilis, S.aureus, P.aerogenosa, B.subtillis, that Pimento dioica* possess compounds containing antibacterial properties that can effectively suppress the growth when extract using acetone as the solvent.

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EFFECT OF MEDICINAL PLANT EXTRACT ON MDR *E. COLI* ISOLATED FROM MILK AND MILK PRODUCT SOLD IN LATUR CITY

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ABSTRACT: *E. coli is a member of coliform group of bacteria. Itis an indicator of fecal pollution of water. It occurs as normal flora in the lower part of intestine of warm blooded animals. It is a commensal organism and opportunistic pathogen. It causes diarrhea, urinary tract infection & nosocomial infection including septicemia & meningitis. This problem is further complicated by presence of antibiotic resistant E. coli. In recent years, drug resistance among human pathogenic bacteria has been commonly reported throughout the world. Bacteria become resistant to antibiotic by different ways. The resistance of bacterial pathogens to many antibiotics continues to increase globally. Patients suffering from antibiotic resistant strain fail to respond antibiotic treatment. So there is a continuous demand of new drugs. In the present study antibacterial activity of Curcuma longa , Elettaria cardamomum and Zingiber officinale was studied against MDR E .coli isolated from milk and milk product sold in Latur city. Antibiotic susceptibility test of the clinical isolates was done by using modified Kirby-Bauer disc diffusion method in accordance with the guidelines of the clinical & laboratory standards institute. Interpretation of resistance was based on the NCCLS criteria. The antibacterial activity of solvent extract of plant was done by agar well diffusion method. The most common pattern of multiple drug resistance of isolates of E. coliobserved was ampicillin- chloramphenicol- amoxicillin- penicillinG- clindamycin. It was found that Curcuma longa andZingiber officinale showed moderate antibacterial activity whileElettaria cardamomum showed least antibacterial activityon isolates of E. coli. The problem of drug resistance could overcome by herbal drugs.*

Keywords: *Antibacterial activity, Disc diffusion method, E. coli, milk and milk product, MDR, Plant extract.*

I. INTRODUCTION

Milk is as an excellent medium for growth of microorganim. Milk can be contaminated with several bacteria during milking process from the milking personnel, utensils,water etc used for milking (Rehman et al.,2014).*E. coli* is a member of coliform group of bacteria. It is Gram negative, straight, short rod, occur singly or in pairs. Itis an indicator of fecal pollution of water. It occurs as normal flora in the lower part of intestine of warm blooded animals. It is a commensal organism and opportunistic pathogen. It causes diarrhea, urinary tract infection & nosocomial infection including septicemia & meningitis. This problem is further complicated by presence of antibiotic resistant *E. coli*. Patients suffering from antibiotic resistant strain fail to respond antibiotic treatment. The indiscriminate use of antibiotics has led to an increase in antibiotic resistance in microorganisms (Anderson, 1968; Ananthanarayan and Paniker, 2003). By different ways bacteria become resistant to antibiotic. R-plasmid often contains genes for resistance to several antibiotics (Dubey and Maheshwari, 2003). Plasmid can be transferred between closely related bacterial populations (Pelzar et al, 2010). Most of the *E. coli* stains are harmless and form part of the normal flora of the gastrointestinal tract of animals *(*Aarestrup et al.,2008). Resistant organism is defined as that organism which is not killed by the drugs available in the body after normal dosage. In other words some species are resistant because they lack a susceptible target or they are impermeable to the antimicrobial agent. Due to this it is a major public health problem. The selective process leading to the emergence and maintenance of bacteria resistance to antibiotics are mainly brought about by the incorrect or abusive utilization of antibiotics (Anderson 1968). The presence of MDR bacteria in milk and milk prduct is consideres as an imprtant public health issue. Food contaminated with antibiotic resistant bacteria act as an ideal vehicle for the transmissin of antibiotic resistant strains to human population (Philips et al., 2004). Upto 30 % of the population in develped country suffer from food born diseases every year.While in developing countries up to two million death are estimated per year(WHO,2007).

The application of plants as medicine perhaps dates back to prehistoric period. The application of plants therefore is as old as 4000 to 5000 B.C. In India earliest references of curative properties of plants appear in Rig-Veda which is said to be written between 3500 to 1600 B. C. The rural population in different parts of the world is more disposed to traditional way of treatment (Jain, 2003; Kirtikar. and Basu, 1988). It is estimated that about 80 % of the rural population in developing Asian nation depend on home care and

traditional medicine for major therapies (Jager et al, 1996). The problem of drug resistance could overcome by herbal drugs. Due to this reason now a day the demand of herbal products as therapeutic agents is increasing all over the world. Keeping this view in mind in the present study an attempt was made to isolate MDR *E coli*from milk and milk products sold in Latur city & to study effect of plant extract on it.

II. MATERIALS AND METHODS

2.1. Isolation of *E .coli* from milk and milk product

100 milk and milk product samples (curd, butter milk, shrikhand and paneer) were collected randomly from different areas of Latur city. All samples were collected in clean sterile containers & were transported to laboratory in ice box. Samples were inoculated in separate Mac Conkeys broth tubes. Tubes were incubated at 37 ºC for 24 hours. A loopful suspension from positive Mac Conkeys broth tubes (tube showing acid & gas) was streaked on the surface of NA, EMB & Mac Conkeys agar plates. Plates were incubated at 37 ºC for 24 hours. The suspected colonies were picked up, sub cultured & identified by using different morphological, cultural & biochemical characters (Ananthanarayan and Paniker .2003; Cruickshank et al, 1975;Dubey and Maheshwari, 2014)**.**

2.2. Antibiotic susceptibility test of isolates of *E coli*

Antibiotic susceptibility testing was carried out by Kirby-Bauer's disk diffusion method (Bauer et al, 1966)for drug susceptibility according to National Committee for Clinical Laboratory Standards (NCCL, 1993). Disc diffusion method was performed to identify the antimicrobial susceptibility pattern of isolates of *E. coli* using Muller Hinton agar Himedia, Mumbai (CLSI, 2012). The Muller Hinton agar plates were smeared evenly using with isolates of *E. coli.* This was then impregnated with antibiotic discs using sterile forceps & then gently pressed down onto the agar. Plates were kept at low temperature and then incubated at 37 ºC for 24-48 hrs. Antibiotics used in this study were Amikacin (30mcg), Amoxicillin (20mcg), Ampicillin (10mcg), Chloramphenicol (30mcg),Ciprofloxacin (5mcg), Clindamycin (2mcg), Gentamycin (10mcg), Norfloxacin (10mcg), Oxytetracycline(30mcg), Penicillin-G (10Units) supplied by Hi-Media Laboratories, Mumbai .

2.3. Preparation of solvent plant extract

Fresh and good quality fruits of *Elettaria cardamomum* & rhizome of *Curcuma longa , Zingiber officinale* were procured from the local market. After cleaning the plant materials were chopped into small pieces with clean knife. Then crushed in mortal and pestle by adding few ml of sterile distilled water. Methanol extract of above plant were prepared separately. The obtained liquid extract was subjected to rotary evaporator and subsequently concentrated and stored in refrigerator and tested by using agar well diffusion method (Mishra et al. 2013).

2.4. Antibacterial testing of plant extract

Suspension of isolates of *E coli* was thoroughly mixed with sterile molten nutrient agar and poured into sterile Petri plates under aseptic conditions. After cooling, plates were used for making of well by using sterile cork borer. 0.5 ml of single plant extract was added in each well. Plates were kept in free for diffusion then incubated at 37 ºC for 24- 48 hrs. After incubation, zones of inhibition were measured & noted (Dubey and Maheshwari, 2014; Mishra et al. 2013).

III. RESULT AND DISCUSSION.

100 isolates of *E coli* were isolated from the milk and milk products of Latur City by using Mac Conkeys agar, EMB agar and Nutrient agar and identified according to morphological, cultural & biochemical characters .Pink and typical colonies were developed on Mac Conkeys agar and EMB agar respectively after incubation. Isolates of *E. Coli* were Gram negative, short rods. Most of the isolates of *E. Coli* have fermented glucose, lactose, mannitol, and maltose. It showed Indole +ve, MR +ve, VP –ve, Citrate –ve, H2S –ve, Urease –ve biochemical tests.10 isolates of *E coli* selected randomly whichshowed antibiotic resistance to one or more antibiotic. The MAR index of each isolate was calculated by using Eq. (1). The antibacterial activity of plant extract on isolates of *E. Coli*was studied, zone of diameter were measured & noted. The MAR index of each isolate was calculated by using following formula:

$$\text{MAR Index} = \frac{\text{No. of Antibiotics to which the isolate was resistant}}{\text{Total no. of antibiotics tested}} \quad (1)$$

Table 3.1. Antibiotic resistance pattern in *E. coli* isolates

Isolate of *E. coli*	Amp	Amx	Amp	Chl	Cip	Cli	Gen	Nor	Oxy	Pen	MAR Index
IEPEC01	S	S	R	R	S	I	S	I	S	S	0.2
IEPEC02	S	S	S	R	S	S	I	S	I	R	0.2
IEPEC03	S	R	S	I	S	S	S	S	S	I	0.1
IEPEC04	I	S	R	R	S	S	S	S	S	R	0.3
IEPEC05	I	S	R	S	S	S	S	S	S	S	0.1
IEPEC06	S	R	S	I	S	S	S	I	S	R	0.2
IEPEC07	S	S	R	S	S	R	S	S	I	S	0.2
IEPEC08	S	I	R	R	S	S	S	S	S	S	0.2
IEPEC09	S	S	R	S	S	I	S	S	S	S	0.1
IEPEC10	S	R	S	R	S	S	S	S	S	I	0.2

(R-Resistant, S-sensitive, I – intermediate, Amp-Amikacin,, Amox- Amoxicillin, Amp-, Ampicillin , Chl- Chloramphenicol, Cip-Ciprofloxacin, Cli - Clindamycin ,Gen-Gentamycin , Nor- Norfloxacin , Oxy-Oxytetracycline, Pen- PenicillinG).

Table3.2. Percent resistance of *E. coli*isolates against individual antibiotic

Sr. No.	Antibiotics	No of isolate showing resistance	Percent resistance
1.	Amikacin(30mcg)	00	00
2.	Amoxicillin(20mcg)	03	30
3.	Ampicillin(10mcg)	06	60
4.	Chloramphenicol(30mcg)	05	50
5.	Ciprofloxacin(5mcg)	00	00
6.	Clindamycin(2mcg)	01	10
7.	Gentamycin(10mcg)	00	00
8.	Norfloxacin(10mcg)	00	00
9.	Oxytetracycline(30mcg)	00	00
10.	Penicillin-G(10Units)	03	30

The most common pattern of multiple drug resistance of isolates of *E. coli*observed was ampicillin-chloramphenicol- amoxicillin- penicillin G- clindamycin.

Table 3.3 Antibacterial activity of plant extract on isolates of *E. coli*

Sr. No	Isolate of *E. coli*	Zone Diameter in mm		
		Curcuma longa	*Elettaria cardamomum*	*Zingiber officinale*
1.	IEPEC01	--	+	+
2.	IEPEC02	++	--	+
3.	IEPEC03	+	--	--
4.	IEPEC04	++	+	++
5.	IEPEC05	--	--	++
6.	IEPEC06	+	+	--
7.	IEPEC07	--	--	+
8.	IEPEC08	+	--	--
9.	IEPEC09	++	+	+
10.	IEPEC10	+	--	--

(--: no antimicrobial activity, +: antimicrobial activity)

It was found that *Curcuma longa andZingiber officinale* showed moderate antibacterial activity *whileElettaria cardamomum* showed least antibacterial activityon isolates of *E. coli. Curcuma longa* shows antimicrobial, hepatoprotective, antioxidant, antitumor, wound healing and antiviral activity (Naz et al,2010). Ginger contains besides some monoterpens, the sesquiterpene-gingibrine alcohol- gingiverol.

Gingerol is very pungent, yellow, a mixture of alcohol. Zinger is traditionally used for a number of gastrointestinal disorders including diarrhea (Nadkarni, 1976). In medicine *Elettaria cardamomum* is used as powerful aromatic, antiseptic, stimulant, antispasmdic, stomachic agent (Korikontimath et al,1999). Medicinal plants are used in many parts of the world for the treatment of bacterial, fungal and viral diseases (Nadkarni, 1976). Plants are rich in secondary metabolites such as tannins, alkaloids terpenoids, flavonoids etc which are antimicrobial, antiparasite and anticancer in nature (Arora and Kaur, 1990; Jain, 2003; Kirtikar and Basu, 1988; Thomson, 1978). The indiscriminate use of antibiotics has led to an increase in antibiotic resistance among microorganisms. Medicinal plants have an important role against microorganisms, they are natural, chief and there is no problem of drugs resistance among bacteria. Due to this reason now a day demand of herbal drugs going on increasing throughout the world.

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BIODEGRADATION OF TEXTILE DYE-CONGO RED

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ABSTRACT: *Congo red, azo dye is commonly used in textile industries for dying of cloths. During the dying process most of the dye is lost in waste water. The dye itself and its metabolites are found to be carcinogenic and mutagenic. The objective of present study was based on degradation of Congo red in laboratory scale bioreactor by using fungi (Aspergillus niger). The degraded dye sample was monitored by UV-VIS and FT-IR analysis. The metabolites obtained after degradation of dye were characterized by LC-MS analysis. The biodegradation of dye using Aspergillus niger showed 92.93% decolorisation after seven days in laboratory scale bioreactor.*

Keywords: *Congored, Aspergillus niger, biodegradation, UV-VIS, FT-IR, LC-MS analysis*

I. INTRODUCTION

Waste water generated through textile industries are generally discharged into natural water resources leading to water pollution. Azo dyes are widely used in textile industries which are synthetically produced organic molecules and represent the largest group of commercial dyes[1]. The chemical structure of these compounds are aromatic rings that are joined by one or more azo groups (-N=N-) have aromatic amines are mutagenic and carcinogenic[10]. These are discharged into water bodies, which reduce light penetration and oxygen transfer into water. These are harmful for human's health, animals, aquatic flora and fauna[8]. The dye used in this study is Congo red, azo dye which is widely used in textile, leather, cosmetics, paper processing industries[6].

Congo red is an organic compound, sodium salt of 3,3'-(1-1'-biphenyl)-4,4'-diyl)bis(4-aminonaphthalene-1-sulfonic acid) or sodium salt of benzidine diazo-bis-1-sulfonic acid[9]. The Congo red have –N=N- bonds which are responsible for their coloration and when such bond is breaks it loses its coloring property[4]. Removal and degradation of Congo red from textile is quite difficult. Biological treatments can be used to degrade azo dyes. The treatment of textile waste water is essential before discharging the waste water into a natural water bodies[3]. Several methods are used to degrade dye like filtration, coagulation, use of activated charcoal, chemical flocculation, these are quite expensive[11]. Alternative to this biological treatments are available at low cost. The aim of present work is to use potential of *Aspergillus niger* for degradation of Congo red and preparation of laboratory scale bioreactor. Fungi are able to degrade dyes due to the extracellular, nonspecific and nonsteroselective, lignolytic enzyme system[2]. The greater biomass production and extensive hyphal growth have been seen effectively used to degrade textile dye.

II. MATERIALS AND METHODOLOGY

Dyes and chemicals

Diazodye (Congo red), Sabouraud's dextrose agar and Sabouraud's broth were procured from Hi-media.

Isolation and screening of fungi

The fungal strain was isolated from soil sample. Soil sample was collected from textile industrial waste discharge site located at MIDC, Solapur, Maharashtra, India. The collected soil sample was subjected to serial dilution by using distilled water. 0.1ml sample from the dilutions 10^{-3} and 10^{-4} was spread on sterile Sabouraud's agar plates[1]. All plates were incubated at room temperature for 72 hrs. Among the different fungal isolates, the fungal strain giving highest decolourization of dye was selected for further study. Lactophenol cotton blue stain was used for staining of selected fungal isolate[4]. Screening of fungal isolate was carried out under compound microscope at 40X and identified on the basis of morphological characteristics.

Dye degradation experiment

For confirmation, the experiment was carried out in conical flask. Dye solution was prepared by dissolving Congo red in distilled water(0.1gm/litre). Sabouraud's dextrose broth was prepared by using dye 0.1gm/litre of water. Isolated fungus was inoculated in dye solution. It was incubated at room temperature

for 7 days on shaker at 100 rpm. Degradation activity was measured on each day and expressed as percent degradation[4].

Measurement of dye degradation by UV- Visible spectrophotometer

The sample was collected after each 24 hrs of degradation process and filtered through Whatmann's filter paper. The filtered sample was centrifuged at 10,000 rpm for 10 mints. The supernatant was collected. The color intensity of degraded sample was assessed by using UV-visible spectrophotometer at 580nm and percent decolourization was calculated by using following formula[5].

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance-Final absorbance}}{\text{Initial absorbance}} \times 100$$

FT-IRanalysis

The degraded dye sample was monitored by FT-IR spectroscopy. The degraded sample was collected and centrifuged at 10,000 rpm. For this, supernatant mixed with KBr pellet in the ratio of 1:100[1]. The pellet was placed in sample holder and the analysis was carried out in the IR region 1000-5000 cm^{-1}.

LC-MS analysis

It was carried out at Indian Institute of Technology, Mumbai, Maharashtra, India. The degraded dye sample(50µl) was subjected to High Resolution Liquid Chromatography Mass Spectroscopic (HR-LCMS). It was kept in a Nano HPLC Chipcube system coupled with 6550 ifunnel Q-TOFs Liquid Chromatography- mass spectrophotometer (Agilent Technologies, USA) system. During analysis, C-18 reserved-phase column, 5-95% acetonitrile in water gradient was used. Mass values of collected fractions were detected by High resolutions (HR) electrometry with mass range of 50-1000. For Q-TOFsMS, fragmentor voltage 175V, nebulizer gas with 35psi and ESI in positive mode was used.

III. RESULTS

Isolation and screening of fungus

The fungal isolate was observed under compound microscope. The black colored mycelia growth was observed on Sabouraud's agar medium. Black colored spores with vegetative mycelia, septate branched, colorless hyphae with foot cells were observed under the microscope. Based on structure of hyphae and color of spores it was confirmed as *Aspergillus niger* (Fig.1). The observation of fungi was compared with observation made by Verma *et al*, 2008[12].

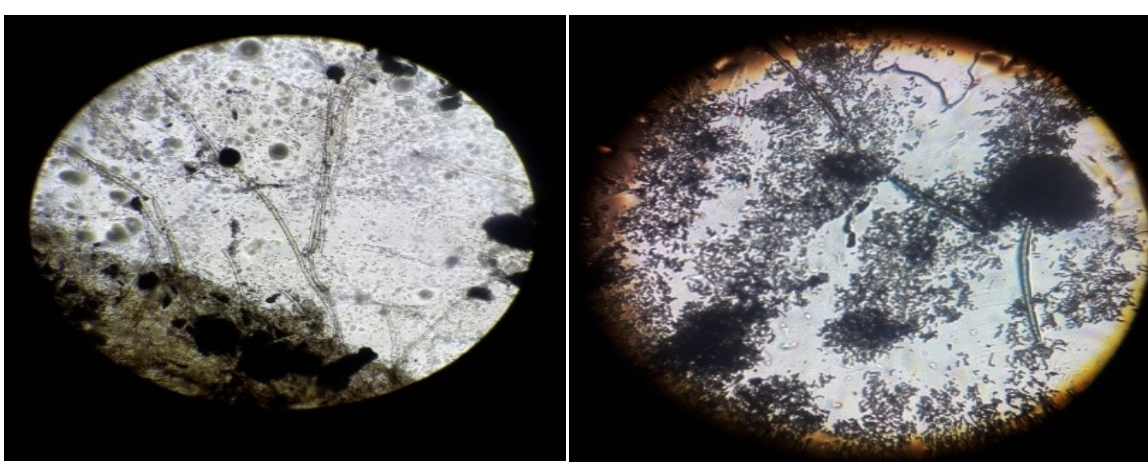


Fig.1: Microscopic observation of fungal isolate (*Aspergillus niger)* under 40*X*

Degradation of Congo red in bioreactor

Continuous stirred tank bioreactor system was used to degrade dye by the fungi *Aspergillus niger*. Overall 0.1 gm/lit dye concentration was used. After 7 days incubation 92.93% broth was decolorized which might be due to absorption and degradation of dye by *Aspergillus niger* (Fig. 2, 3 & 4).

Table1: Cultural conditions for growth of *Aspergillus niger* and dye degradation

Sr. No.	Parameter	Growth of fungus	Dye degradation
1.	Optimum temperature	30°C	30°C
2.	Optimum pH	5.8	5.8
3.	Aeration rate	-	2 vvm
4.	Agitation rate	100rpm	300 rpm
5.	Incubation time	2 days	7 days

In bioreactor 5.8 pH, 300 rpm agitation rate, laboratory aerator for aeration, room temperature was maintained for dye degradation. The culture density was maintained in bioreactor by using continuous removal of mycelia from bioreactor. The spore count of bioreactor was maintained upto 10^8 spores/ml of broth which is determined by heamocytometer[7]. (Table1)

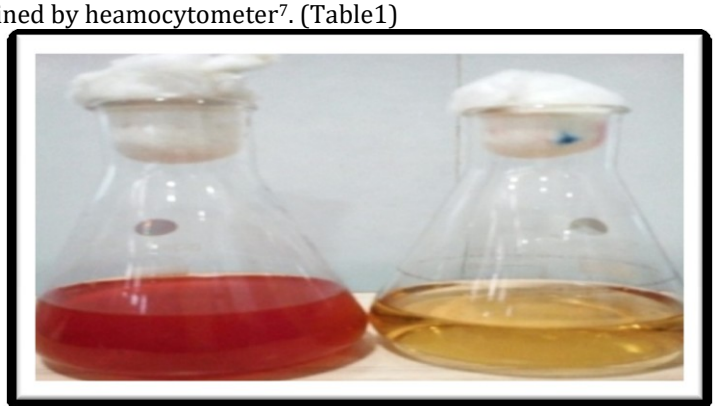
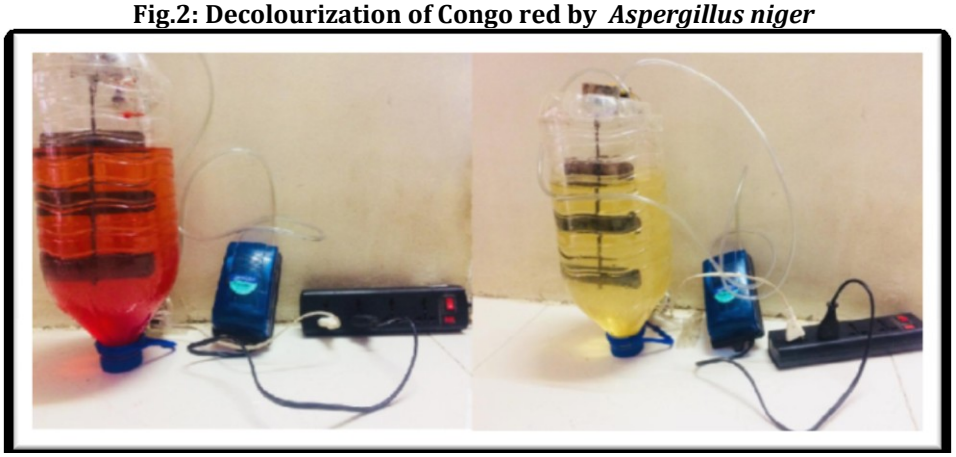


Fig.2: Decolourization of Congo red by *Aspergillus niger*



Before degradation **After degradation**

Fig.3: Laboratory scale bioreactor

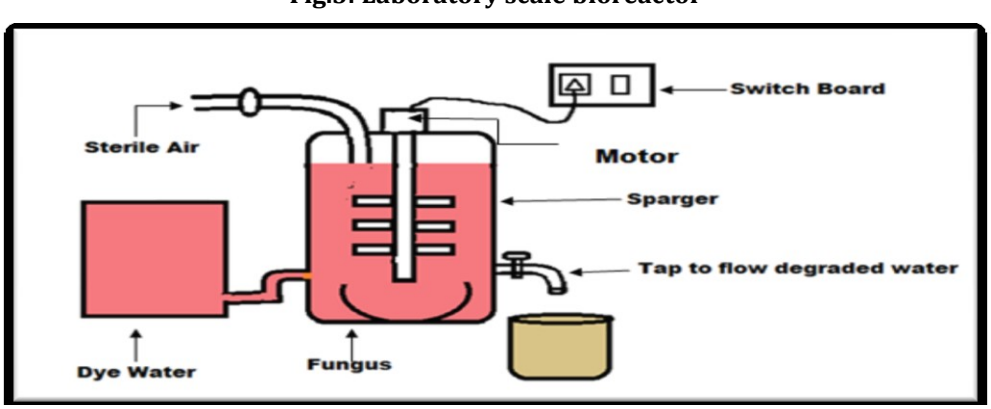


Fig.4: Schematic representation of pilot scale bioreactor

Percent degradation of dye

Efficacy of *Aspergillus niger* was evaluated for decolourization of Congo red in Sabouraud's broth (0.1gm/L). The degradation of Congo red was found to be 92.93% after 7 days incubation at pH 5.8. The colour was changed from red to colourless. (Table2) (Fig.3)

Table2: Percent decolourization of degraded dye sample

Sr.No.	Time in hours	O.D. at 580 nm	Percent Decolourisation
1	24	0.21	39.30
2	48	0.12	65.37
3	72	0.10	69.73
4	96	0.07	77.96
5	120	0.07	78.51
6	144	0.07	79.76
7	168	0.02	92.93

FT-IR analysis

FT-IR spectra of Congo red and decolourized sample resulted in biodegradation (Fig.2). The FT-IR spectrum of Congo red was showed that functional group region at 2000-1600cm^{-1} for C-H stretching bond. The functional group region was observed at 3200-3400cm^{-1} for presence of N-H stretching bond. The FT-IR spectrum of decolorised sample by *Aspergillus niger* showed finger print region at 1000-500cm^{-1} for C-O stretching bond. Functional group region were found at 2260-2100cm^{-1} for presence of C=C stretching bond and 3500-3450cm^{-1} for N-H stretching vibration.Observation of various functional groups after FT-IR analysis of decoloured Congo red sample showed that it was degraded into various compounds.(Fig.5)

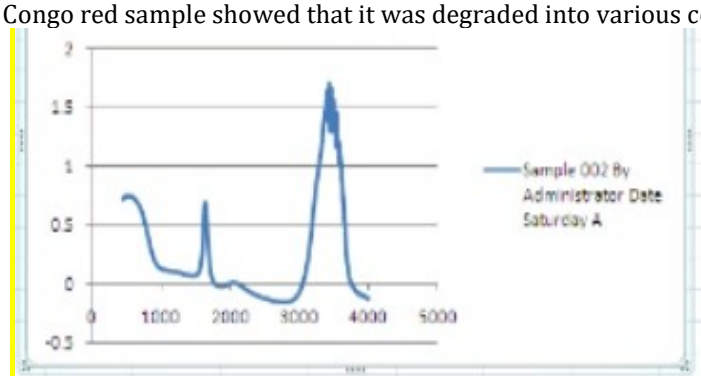


Fig.5: FT-IR Spectra analyses dye sample before and after degradation

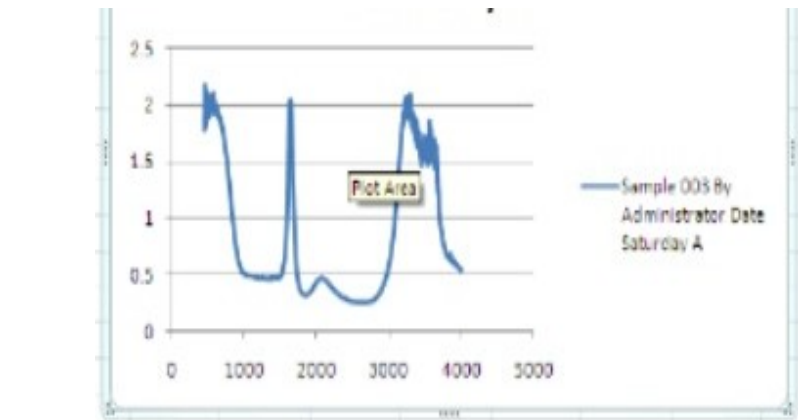
LC-MS analysis

LC-MS analysis oftreated dye sample showed different patterns of compounds at different m/z ratios after 72 hrs.(Fig.6a,6b) The product of degraded dye sample were identified at different retention time (Table 3). Present data in table 3 indicates that Congo red dye was degraded into various low molecular weight compounds.

Table 3: Metabolites analysed after LC-MS analysis of degraded Congo red sample

Sr.No.	Retention time(min)	m/z	Metabolites	Formula
1	0.904	296.1581	Lactone of PGF-MUM	$C_{16}H_{24}O_5$
2	1.13	133.0514	4-Hydroxy benzyl cynide	C_8H_7NO
3	1.246	211.0943	Zalcitabine	$C_9H_{13}N_3O_3$
4	1.437	189.1124	Homocitrulline	$C_7H_{15}N_3O_3$
5	1.515	132.0911	DL-Ornithine	$C_7H_{12}N_2O_2$
6	1.523	135.0541	Adenine	$C_5H_5N_5$
7	1.344	330.1892	SerLysPro	$C_{14}H_{26}N_4O_5$
8	3.234	123.0682	2-Amino-p-cresol	C_7H_9NO

9	5.066	226.1307	Amobarbital	$C_{11}H_{18}N_2O_3$
10	5.15	290.1371	Trimethoprim	$C_{14}H_{18}N_4O_3$
11	7.52	268.1204	Isobutyglycine	$C_{11}H_{16}N_4O_4$
12	9.289	567.2893	Dihydrodeoxystreptomycin	$C_{21}H_{41}N_7O_{11}$
13	10.318	546.2646	ArgTrpTrp	$C_{28}H_{34}N_8O_4$
14	10.677	160.0751	Hydralazine	$C_8H_8N_4$
15	10.913	287.2812	C17 Sphinganine	$C_{17}H_{37}NO_2$
16	15.958	310.1548	Avobenzone	$C_{20}H_{22}O_3$



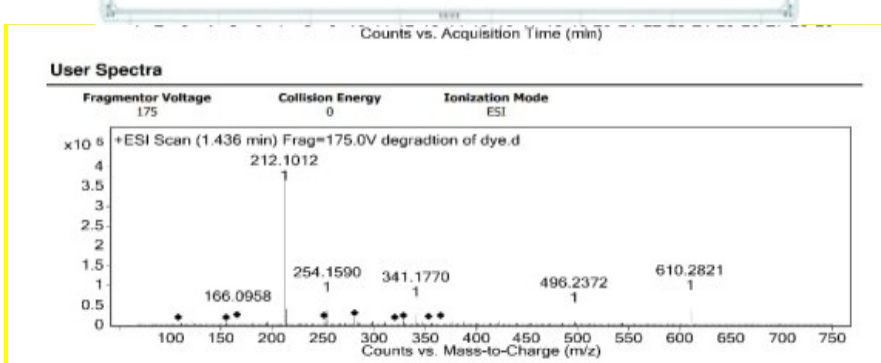


Fig 6a: LC-MS Spectrum of Congo red degraded product formed during Congo red decolourisation by *Aspergillus niger*

Fig 6 b: LC-MS data of Mass to charge ratio obtained after Congo red degradation by *Aspergillus niger*

III. DISCUSSION

The reactive orange 16(RO-16) was degraded upto 63.50% to 69.27% respectively by using packed bed reactor containing Ashoka and Casurina seeds[11]. Viscose orange-A dyewas degradedup to 88.70% by *Aspergillus funigatus*[4]. FT-IR spectrum of metabolites obtained after decolonization of Bismark Brown Y by *Alternaria Brassicae* showed cleavage of azo bond[1]. FT-IR results of degraded sample of Congo red by *Aspergillus niger* showed presence of phenolic compounds and LC-MS spectra of Congo red dye showed presence of benzene ring, sodium naphthalene sulfonate, cyclopheptadienylium at different m/z ratios[2].

Observations of these findings gave support for use of *Aspergillus niger* (fungus) for degradation of Congo red effectively.

IV. CONCLUSION

In presence study Congo red dye decolourization was found effective with using continuous stirred tank bioreactor under aerobic condition. Use of *Aspergillus niger* for dye degradation proved to be effective in 92.93% degradation of dye after 7 days. LC-MS analysis revealed formation of different metabolites after degradation of dye sample UV-VIS, FT-IR, LC-MS analysis have proven the effective role of *Aspergillus niger*

in degradation of Congo red. Further study can be extended to degrade metabolites obtained after dye degradation.

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MICROBIAL PRODUCTION OF BIOACTIVE COMPOUND (VITAMIN B12) FROM POND SEDIMENT

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ABSTRACT: *A bioactive compound is present in the foods of humans, animals, that has an effect on the organism consuming it. Pond derived organic species were isolated from the pond sediment and identified by the morphology biochemical analysis. The batch fermentation process was used for the production of vitamin B12 at optimal conditions with specific fermentation medium. Pond derived species isolates, produced vitamin B12. The concentration was later determined by UV-VIS Spectrophotometer. The standard sample was the commercially available cynocobalamine that is dosage form of VitaminB12. The fermentation leads to the production of the Vitamin B12 in Bennett media.*

I. INTRODUCTION

Water microbial technology has opened a wide area for finding novel organisms for trapping their potentiality. The microorganisms growing in water environments are metabolically and physiologically diverse from terrestrial organisms. There are some novel and valuable source of new bioactive compounds. Vitamins are regarded as organic compounds required in the diet in small amounts to perform specific biological functions for normal maintenance, optimum growth and health of the organisms. Vitamin B12 (cobalamin, anti-pernicious anemia factor) is a water soluble vitamin with a key role in the normal functioning of the brain and nervous syste m and for the formation of blood. It is normally involved in the metabolism of every cell of the human body, especially fatty acid synthesis and energy production. It is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation synthesis. It is a unique vitamin, synthesized by only microorganisms and not by animals and plants. Vitamin B12 has been isolated from fermentation broths of numerous microorganisms, including antibiotic-producing organisms. Industrial production of Vitamin B12 is through fermentation of selected microorganisms .Determination of vitamin B12 production was carried out using the microbial species isolated from the pond sediment is a common ingredient in energy drinks and energy shots, usually at several times the minimum recommended daily allowance of B12.c

II. MATERIALS AND METHODS

1. SAMPLE COLLECTION

The pond sediment sample was collected from pond of Walchand college Solapur Maharashtra, India at 5 m depth in sterilized glass bottle along with some water and its sediments.

2. PREPARATION OF MARINE SEDIMENT SUSPENSION AND SERIAL DILUTIONS

20 grams of pond sediment was transferred into 250 ml conical flask containing 100 ml of sterilized physiological saline and this sample was serially diluted up to 10 - 4 dilution. Each test tube of diluted samples were vortexed vigorously for 15 minutes

3. PREPARATION OF BENNETT MEDIA

Bennett Media composition

S.NO INGREDIENT QUANTITY 1. Beef extract 1g 2. Yeast extract 1g 3. Casein digest 2g 4. Agar 17g 5. Glucose solution (25% w/v) 20ml 6. Maltose solution (25% w/v 20ml 7. Nystatin solution (0.25%w/v) 20ml 8. Nystatin solution (0.25%w/v) 20ml 9. Double distilled water 470ml 10. Pond water 470ml After adding the ingredients, the pH was adjusted to 7.4 before autoclaved.

2. ISOLATION OF COLONIES :

The serially diluted samples were spread evenly on the media and kept it for incubation at 37°C for 96 hours. After incubation, different species were isolated by morphological methods.

4. INOCULUM PREPARATION

The suspension was prepared for each strain and 24 hours old slant culture was used. The inoculum was prepared by transferring 5ml of suspension into 100ml conical flask containing 45ml of sterile inoculum medium. 50% of sea water and equal volume of double distilled water was used to prepare the

inoculum. Then it was kept in electric shaker with 100 rpm for 24 hours. The medium described by Tanaka et al., 1974(16) was modified and contained

NH4PO4 - 0.2%
KH2PO4. - 0.2%
CaCl2.2H2O - 0.001%
FeSO4.7H2O - 0.003%
MnSO4.n H2O - 0.002g
CoSO4.7H2O - 0.005%
0.5 g glucose per 50ml.

4. FERMENTATION PROCESS

Fermentation studies were carried out in the conical flasks. Fifty milliliters of 24 hours aged inoculum at the ratio of 10% (v/v) was added to 200 ml of production medium in 250 ml of conical flask. The glucose was used as a carbon source. The pH of culture was adjusted to 7.4 and the fermentation was carried out at 300C for 5 days. The growth of bacterium and vitamin B12 production was measured. The fermentation medium used contained(g/l)

NH4PO4 - 2g
KH2PO4 - 2g
CaCl2.2H2O. - 0.01g
FeSO4.7H2O, - 0.005g
MnSO4.n H2O, - 0.005g
CoSO4.7H2O. - 1.0mg
CoSO4.7H2O - 1.0mg
The glucose was used as a carbon source.

5. EXTRACTION OF VITAMIN B12

The extraction of vitamin B12 was done by harvesting the cells from fermentation broth and centrifuged at 10,000 rpm at -4'C for 10 minutes. The pellets were washed with 0.2M potassium phosphate buffer (pH 5.5) and suspended in the same buffer containing 0.1% of KCN. The suspension was autoclaved for 15 minutes at 121'C. The supernatant containing extracted vitamin B12 was filtered through a cellular acetate membrane filter with 0.2µm and stored at -4'C.

6. ESTIMATION OF VITAMIN B12

The vitamin B12 was estimated by UV-VIS Spectrophotometer (Double beam, I-1029) with optimum absorbance of vitamin B12 (360nm) with 0.2M Potassium Phosphate Buffer as reference. Production of vitamin B12 was estimated for each isolated Streptomyces species. The concentration of vitamin B12 was quantified with the help of standard graph and the formula. (Test O.D / Standard O.D) × Concentration of standard solution.

III. RESULT

Glucose utilizing 18 species were isolated from pond sediment based on the morphology of growth, colour and appearance.

VITAMIN B12 CONCENTRATIONS

Few microorganisms producing significant amount of vitamin B12 eighteen isolates produced vitamin B12 among which S15 (strain no. 15) produced remarkably high amount (3.08 µg/ml) of vitamin B12. Other species produced vitamin B12 in the range of 0.35 to 3.08 µg/ml.

Strain number
Concentration of Vitamin B12(µg/ml)
S1 S3 S4 S9 S11 S15 S16 S17
0.96 0.92 1.21 0.92 0.8 3.08 1.04 1.33

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SYNTHESIS OF STABLE ANALOGS OF ORGANOSULPHUR COMPOUND (ALLIIN) FROM *ALLIUMSATIVUM L* AS POTENT ANTI-MICROBIAL

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ABSTRACT: *Mannich bases and alliin individually show various anti infective activities such as antimicrobial, antifungal, anticonvulsant, antimalarial, analgesic and anti-inflammatory type of varied pharmacological activities. If isolation of alliin is tried, it leads to decomposition and inactivation. Hence if to be used in isolated form it has to be converted into stable form. The novelty of present research work is synthesis of mannich bases of alliin as stable semisynthetic analogs. As alliin and mannich bases individually show various type of pharmacological activities, one of which anti-infective activity, combined analogs should show synergistic activity. In first step synthesis of mannich bases is done using aldehydes, ketones and amines having aliphatic, aromatic and heterocyclic nature. Synthesized mannich bases were condensed with alliin to get mannich base derivatives of alliin. The confirmation of antimicrobial activity of synthesized analogs is carried out by BHI microbial assay method using Escherichia Coli and Staphylococcus aureus.*

Keywords: *anti infective, mannich bases, alliin , mic*

I. INTRODUCTION

Infectious diseases are caused by bacteria, viruses, fungi, protozoa, parasites, orprions affect millions of people worldwide. Several high-profile reports have keenly carried out survey for examining infectious diseases and development of resistance and have emphasized measures such as surveillance, infection control, and better stewardship ofexistinganti-infective agentsthroughrational use. Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability and death.

Without effective antimicrobials for prevention and treatment of infections, is going to be challenging task. Hence there is continuous and urgent need for discovery of new potent antimicrobials.[1]
Natural products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity.

In literature there is mention of constituents of *AlliumSativum L* like alliin, which are organosulphur compounds and are known to show antimicrobial activity. If isolation of alliin is tried, it gets inactivated due to decomposition. To overcome the problem, one of the way is chemical derivatization, whereby alliin can be convert into a stable analogs.

In literature survey it is mentioned that mannich bases and alliin individually show various types of pharmacological activities like anticancer, antimicrobial, antifungal, and anti-inflammatory. In present work as novelty it was thought to carry out synthesize mannich base derivatives of alliin as stable analogs.[6-7]

For synthesis of mannich bases, use of mannich reaction is done which is amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine. Synthesized mannich bases were condensed with alliin to form mannich base derivatives of alliin.[9-12]

Determination of minimum inhibitory concentration (MIC) is generally done as the most basic laboratory measurement for determination of activity of antimicrobial agent against an organism. The lower MIC value indicates that less of the drug is going to be required in order to inhibit growth of the organism. With intention to estimate MIC of mannich base derivatives of alliin, screening for anti- microbial activity using Escherichia Coli and Staphylococcus aureus,was done using BHI (brain heart infusion) broth dilution method.[13-21]

II. MATERIALS AND METHODS

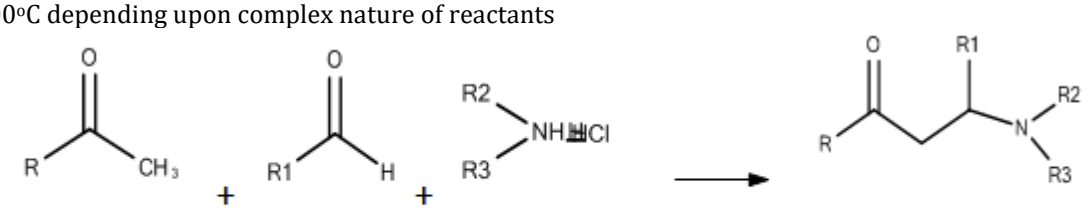
For synthesis of mannich bases in first step use of three reactants like aldehyde, ketones and amines having aliphatic, aromatic, cyclic and heterocyclic nature was done. It second step condensation of synthesized mannich bases was done with alliin to form mannich bases of alliin.[9-12]

SCHEME OF SYNTHESIS

Step-I: Synthesis of Mannich base

Procedure: 1.05-1.10 molecular equivalent of amine was taken in flat bottom flask and converted to hydrochloride salt using concentratedhydrochloric acid, formation of salt was determined by use of Congo red paper. To this was added 1.00 molecular equivalent of carbonyl compound i.e. ketone and 1.5-2.0 molecular equivalence of aldehyde.

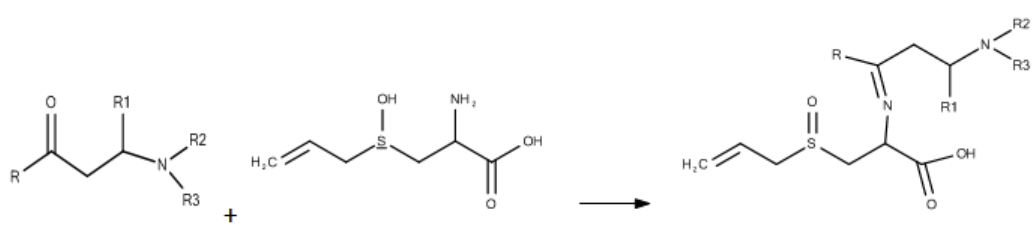
Reaction conditions optimization with respect to time and temperature had to be done for each individual reaction. Time required was found to vary from 30 minutes to 12-14 hours, with temperature conditions varying from room temperature with mechanical stirring, to heating on water bath at temperature between 80-100°C depending upon complex nature of reactants



Aldehyde Ketone Amine Mannich Base

Step-II: Synthesis of Mannich bases of Alliin

It is a simple condensation reaction, where mannich bases synthesized in step-1 are treated with approximate one mole quantity of alliin in presence of alcohol as solvent and refluxing reaction mixture on water bath for around half an hour. Synthesized compounds are shown in table no 1.



Mannich Base Alliin Mannich bases of alliin

Step-III: Estimation of anti microbial[13-21]

Estimation of antimicrobial activity was done using BHI (brain heart infusion) broth dilution method using *Escherichia Coli* .and *Staphylococcusaureus,* for anti-microbial activity.[13-19]

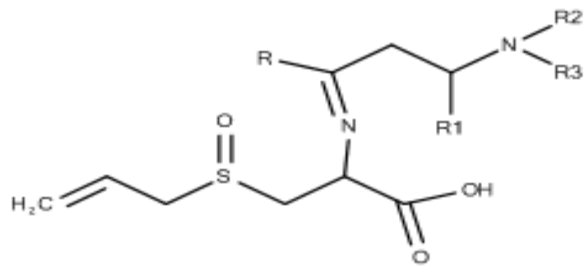
The results for antimicrobial activity are shown in table no. 2

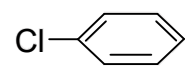
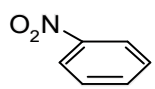
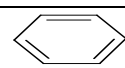
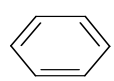
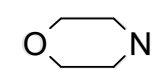
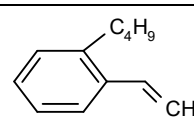
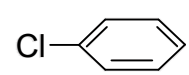
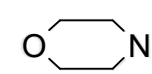
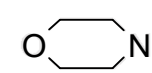
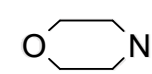
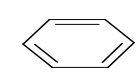
III. RESULTS

Depending upon the complexity of structure of three reactants, optimization of reaction conditions with respect to time and temperature had to been done on individual basis. Time span of reaction varied from 30 minutes to12-14 hours. Optimum temperature condition were optimized for each reaction and varied from room temperature with mechanical stirring to temperature between of 80-100°C. Percentage yield varied from 24 to 73 %. Structure of synthesized analogs are mentioned in table no. I

Estimation of antimicrobial and antifungal activity was done using BHI (brain heart infusion) broth dilution method using Escherichia Coli (8739) Staphylococcus aureus (25923). The results of activity are mentioned in table no. II

Table No I : Synthesized Compound



Compound	R	R1	R2	R3
1	CH_3	H	C_2H_5	
2	C_2H_5		C_2H_5	
3		H	C_2H_5	
4		H	C_2H_5	
5	CH_3CH_2	$CH_3CH_2CH_2$	CH_2CH_2OH	
6		H		
7				
8	CH_3	H		
9	$CH_3CH_2CH_2$	H		
10	CH_3	H		

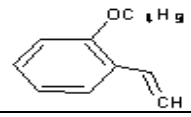
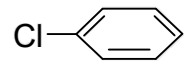
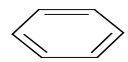
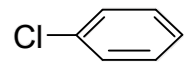
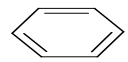
11			
12	C_6H_5-CH=CH		

Table No II: Results for antimicrobial activity

Sr.No	Compound No	Activityon *S.aureus* MIC(µg/ml)	Activityon *E.coli* MIC(µg/ml)
1	1	16.60	500
2	2	16.60	500
3	3	16.60	500
4	4	16.60	500
5	5	62.50	62.50
6	6	250	250
7	7	125	250
8	8	250	250
9	9	250	250
10	10	16.60	16.60
11	11	62.50	16.60
12	12	62.50	16.60

IV. DISCUSSION

Complexity of aldehyde, ketones and amines plays a significant role in deciding time, temperature and % yield of synthesized products.

The structural diversities present in microorganisms plays a significant role in showing activity. On the basis of results of activities carried out following conclusions are drawn,

(1) Alkyl derivatives of alliin are found to be more active against *Staphylococcus aureus*than *Escherichia Coli.*

(2) Alkyl derivatives of alliin were found to be more active against *C. albicans*Compared to *Apergillusniger*.

(3) Acyl derivatives are found to be partly active.

(4) Mannich bases of alliin formed from aliphatic carbonyl compounds has shown good anti-microbial and anti-fungal activity against *Staphylococcusaureus*and *C. albicans*.

(5) Unsubstituted aromatic ring containing alliin derivatives were found to be more active than 3 and 4-substituted aromatic ring derivatives.

(6) Highest activity was shown by mannich bases of alliin which have morpholine as amine with one of the other component having aromatic nature.

(7) Alliin derived from aromatic ketones are active although somewhat less active than the alliin derivatives of corresponding aldehyde.

(8) Alliinderived from heterocyclic aldehydes also show comparable activity to alkyl derivatives.

V. CONCLUSION

Synthesized compounds inhibit the growth of microorganism by inhibiting cell wall synthesis. Hence depending upon the composition of microbial cell wall it is found that different compounds with

different structural features show varied activity as discussed. Structural complexicity plays significant role in time required, temperature conditions and % yield of compounds synthesized.

VI. ACKNOWLEDGEMENT

Author is thankful to Principal, Dr.H.N.More, Mr. R. P. Dhavale , BharatiVidyapeeth College of Pharmacy, Kolhapur for providing excellent facilities to carry out synthetic and anti-microbial activity work.

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OPTIMIZATION OF NATURAL SUBSTRATES FOR XYLANASE PRODUCTION USING *ASPERGILLUS NIGER* AS A FERMENTING ORGANISM

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ABSTRACT: *Xylanase constitute one of the most important kind of enzyme which is extensively used in industries namely wood and pulp industries, baking industries, pharmaceutical industry , food and feed industry.Aspergillusnigeris source of xylanase production and is economically fesiable for production of enzyme.Xylanase production can be done by Solid State fermentationby solid substrate likely wheat bran , sugarcane baggase, saw dust ,and paddy straw.And Optimization of Temperature , Optimization of pH , Optimization of Carbon sources , Optimization of Nitrogen sources for production of Xylanase was carried out.Among the various substrates studied Saw dust was found to be the best substrate for Xylanaseproduction.The Optimum temperature for Xylanase production was found to be 30^{0}C. The Optimum pH for Xylanase production was found to be 7. The Optimum Carbon source for Xylanase production is Lactose. The Optimum Nitrogen source for Xylanase production is Yeast Extract.The yield of enzyme under Optimum condition was found to be 2.68 IU/ml.*

Keywords: *Xylanase production , Solid State fermentation.*

I. INTRODUCTION

Enzymes are proteins that accelerate and mediate the rate of biochemical reactions in the living sstem.The enzymes are broadly classified into two category based on their secretion as extracellular enzymes and intracellular enzymes. The intracellular enzymes which will be secreated inside the cell and governing the cell metabloism . Whereas the extracellular enzymes which hydrolyze the coplex organic phosphates in to inorganic phosphates by the process dephosphorylation which will be further used by the cell to construct the Nucleic acid , Phospholipids , ATP energy molecules etc..

Like all proteins, enzymes are he long , linear chains of aminoacids that fold to produce a three dimensional product. The building blocks of proteins are amino acids ,which are small organic molecules that consist of an alpha (central) carbon atom linked to an amino group , a carboxyl group , a hydrogen atom and a variable component called a side chain. Within a protein ,multipleaminoacids are linked together by peptide bonds thereby forming a long chain . Peptide bonds are formed by a biochemical reaction that extracts a water molecule as it joins the aminogroup of aminoacid to the carboxyl group of a neighbouringamino acids. Each unique aminoacid sequence produces a specific structure, which has unique properties. Individual protein chains may some times group together to form a protein complex.

Enzymes are in general globular proteins and range from 62 aminoacid residues in size to over 2500 residues in the animal fatty acid synthase. The activities of enzymes are determined by their three dimensional structure . Most enzymes can be denatured that is unfolded and inactivated by the heating or chemical denaturants , which disrupts the three dimensional structure of the protein . Depending on the enzymes , denaturation may be reversible or irreversible . Enzymes are usually very 6 specific as to which reactions that catalyze and the substrates that are involved in this reactions .a few enzymes exhibit absolute specificity , that is they will catalyze only in the particular reaction. Other enzyme will be specific for a particular type of chemical bond or functional group .In general , there are four distinct types of specificity :

1. Absolute specificity : The enzymes will catalyse only one reaction.
2. Group specificity : The enzymes will act only on molecules that have specific functional groups such as amino , phosphate and methyl groups.
3. Linkage specificity : The enzymes will act on a particular type of chemical bond regardless of the rest of the molecular structure.
4. Stereochemicalspecificity : The enzymes will act on a particular steric or optical isomer.

Enzymes can also show impressive levels of stero specificity and chemoselectivity . Most enzymes are much larger than the substrates they act on and only a small portion of the enzyme is directly involved in catalysis . The region that contains these catalytic residues , known as the active site , binds the substrate

and then carries out the reaction . Enzymes can also contains sites that bind cofactor that are needed for catalysis. Some enzymes also have binding sites for small molecules , which are often direct or indirect products or substrates of the reaction catalyzed. This binding can serve to increase or dercease the enzyme activity , providing a means for feedback regulation . Some enzymes that produce secondary metabolites are described as promiscuous , as they can act on a relatively broad substrate specificity is important for the evolution of new biosynthetic pathways.

Specificity of enzymes may be attributed to the fact that , both the enzymes and the substrate possess specific complementary geometric shapes that fit exactly into one another .This is often referred to as "The Lock and key " model. However while this model explains enzyme specificity it fails to explain the stabilization of the transition state that enzymes achieve.

Xylanase :

Xylane is a heteropolysaccharide containing substituent groups of acetyl ,4-o-methyl-D-glucuronosyl and alpha – arabinofuranosyl residues linked to the backbone of beta – 1,4,- linked xylopyranose units and has binding properties mediated by covalent and noncovalent interactions with lignin , cellulose and other polymers. Lignin is bound to xylans by an ester linkage to 4-o-methyl-D-glucuronic acid residues. The depolymerization action of endoxylanase results in the conversion of the polymeric substances into xylooligosaccharides and xylose. Xylanases are fast becoming a major group of industrial enzymes finding significant application in paper and pulp industry. Xylanases are of great importance to paper and pulp industry as the hydrolysis of xylan facilitates release of lignin from paper and reduces the level of usage of chlorine as the bleaching agent.

Xylan, one of the major components of hemicelluloses found in plant cell wall is the second most abundant polysaccharide next to xylose.The term hemicelluloses refer to the plant cell wall polysaccharide next to xylose . The term hemicelluloses refers to the plant cell polysaccharides that occur in close association with cellulose and glucans .in fact the plant cell wall is a composite material in which cellulose , xylan and lignin are closely linked . Xylan having a linear back bone of beta -1,4- linked xyloses is present in all terrestrial plants and accounts for 30% of the cell wall material of annual plants 15- 30% of hard woods and 7- 10% of soft woods.

Xylanase is the name give to a class of enzymes which degrade the linear polysaccharide beta -1,4-xylan into xylose , thus breaking down hemicellulose , one of the major components of the major components of plant cell walls.

Xylanases are extracellular enzymes produced by microorganisms such as bacteria (saprophytic and phytopathogenous) , Mycorrhizic fungi and some yeasts . The enzymes is also found in protozoa, insects, crustaceans ,snails , seaweed and also seeds of plants during the germination phase in the soil.

II. MATERIAL AND METHODS

Material

Soil sample was collected from garden of Krishna Institute of Medical sciences Deemed University Karad .

Methods

1. Isolation of Xylanolytic fungi

The isolation of fungi was carried out on Potato Dextrose Agar (PDA) using the samples obtained from agricultural soil. 1.0 gram of soil sample was suspended in 10ml of distilled water and shaked vigorously for 10 min.Then 0.5ml of diluted suspension was spread on PDA using an L-shaped rod and incubated colonies were observed under the identify the specific based on the following identification and characteristic features. The fungal isolates formed were subcultured to purify and examined for xylanolytic activities.

Identification of fungus :

Identification of fungus was done by observing colony characteristics and microscopic examination.

Lacto phenol cotton blue staining :

A loopful of isolate was placed on the clean glass slide , a drop of lacto phenol cotton blue stain was then mixed with the culture. A clean cover slip was placed over surface and viewed under the microscope (45X) and the morphology of isolate was observed.

2. Solid State Fermentation (SSF)

Cultivation of fungus was performed in 250ml of Erlenmeyer flask containing 10g of solid substrate such as wheat bran , sugarcane baggase , saw dust and paddy straw with the addition of 15ml Mendel's medium. The medium was autoclaved and inoculated with spores of moistening agent and incubated for 5

or 7 days at the ambient temperature 30^0 C.

3. Enzyme extraction

70ml of cold water (4^0C) was added to the SSF medium (10 gm substrate) after cultivation. The mixture was centrifuged at 5000rpm for 20min.The solid biomass residues were separated from the suspension by the filteration through Whatmann filter paper .The cell free supernatant was used as the source of crude enzyme preparation.

4. Alkaline extraction of xylan

Alkaline extraction of xylan from lignocellulosic sample was performed following a modified protocol. The mild lignocellulosic powder sample (sugarcane baggase) was soked in 10% sodium hydroxide (with ratio of 1:10) and kept overnight in constant agitation at 60^0C , and then were steamed at 100^0C for 3 hours. After alkaline treatment the supernantant was recovered by centrifugation (10,000 rpm for 15 minutes) was acidified with 12N Hcl to pH 5.0. Then 1.5 volume of 95% ethanol was added to precipitate the xylan.Aftercentrifugation , the xylan was allowed to air dry before drying in hot air oven for 4 hours at 55^0C. The pellets were weighted and powdered for using as a substrate for the production of xylanase. The true recovery of xylan from agrowaste is given here.

True recovery(%) = Dry weight of extracted xylan
Weight of sample (g) × 100

$$= \frac{9.2}{20 \times 100}$$

= 46 %

Therefore True recovery of Alkaline extraction of Xylan is 46 %.

5. Optimization of fermentation media (Saw dust) at different temperatures, pH , carbon sources, and nitrogen sources

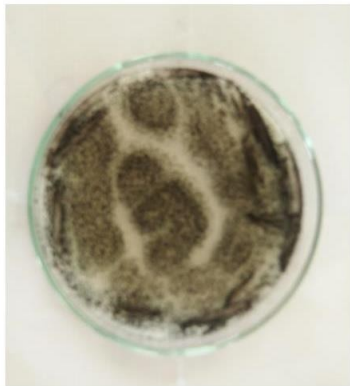
Optimization of fermentation media (saw dust) was done by inoculating flask containing media with different temperature ranges 25, 30,37 and 40^0C , pH range (pH 6 to 9), carbon sources such as glucose, lactose, xylose, maltose of carbon source at 1% concentration and nitrogen sources such as peptone ,urea, yeast extract, NaNO3 of nitrogensoures at 0.075% concentration

III. RESULT AND DISCUSSION

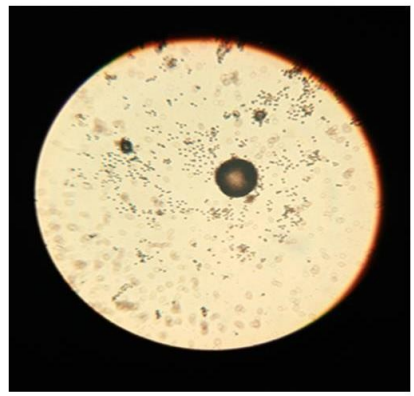
The four fungal isolates were isolated from garden soil sample are designed as XF1,XF2,XF3, and XF4 and subjected to screening of xylanolytic activity using Malt Extract Agar (MEA) . The fungal isolate XF3 which showed maximum xylanolytic activity was selected for further study.The isolate XF3 was characterized with respect to its morphological and cultural characteristics and was tentatively identified as a strain of *Aspergilusniger*.

Production of xylanase activity was carried out using Mendal'smedium . Optimization study regarding the optimization of substrate (Wheat bran, Sugarcane baggase, saw dust and paddy straw) was carried out. Among the various substrate for xylanase production .Optimization of temperature, optimization of pH optimization of carbon sources and optimization of nitrogen sources for the production of xylanase was carried out.

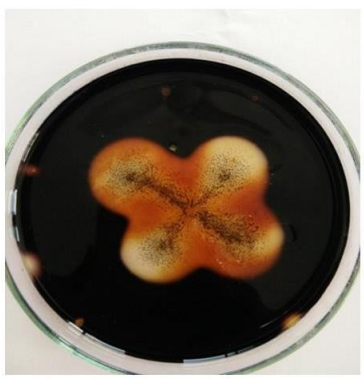
The optimum temperature for xylanase production was found to be 30^0C.The optimum pH for xylanase production was found to be 7. The optimum carbon source for xylanase production was found to be Lactose. And optimum nitrogen source for the xylanse production was found to be yeast extract . Enzyme activity was measured in termesof IU/ml. The yield of enzyme under optimum condition was found to be 2.68/ml



Cultural Characterstics of fungal isolates



Morphological Characterstics of fungal isolates



Screening of Xylanolytic producing activity

Optimization of substrate (Solid state fermentation)

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EFFECT OF CARBON SOURCES ON PRODUCTION OF EXOPOLYSACCHARIDE OF HALOALKALIPHILIC ARCHAEON *NATRIALBA WUDUNAOENSIS SSBJUP-5* ISOLATED FROM LONAR LAKE

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ABSTRACT: *An exopolysaccharide producing haloalkaliphilic archaeon Natrialba wudunaoensis SSBJUP-5 was isolated from Lonar Lake situated in Buldhana District, Maharashtra, India. This study was aimed to study the exopolysaccharide (EPS) production by haloloalkaliphilic archaebacteria and to investigate the effect of different carbon sources on its production. EPS have wide applications in food, pharmaceuticals, petroleum and other industries. Amongst carbon sources examined, glucose induced higher polysaccharide production with the yield of 0.525% by mass and 96 µg/mL by chemical estimation at 0.75% concentration of glucose in the medium. This is followed by sucrose and lactose. However maltose and galactose were found poorest carbon sources giving lower yield of EPS.*

Keywords: *Exopolysaccharide, Carbon source, Haloalkaliphilic archaeon, Lonar Lake.*

I. INTRODUCTION

Exopolysaccharides (EPS) are natural, non-toxic, and biodegradable polymers produced by different organisms and play important roles in various biological mechanismssuch as immune response, adhesion, infection, and signal transduction (Kumar et al, 2007). Now a day,a considerableresearch has been directed on biological activity of thesepolysaccharides by structure-function analysis (Sutherland, 1998).Besides the interest on their applications in the health and bionanotechnologysectors, polysaccharides are also used as thickeners, bioadhesives, stabilizers, probiotic,and gelling agents in food and cosmetic industries and as emulsifier,biosorbent, and bioflocculant in the environmental sector (Sam et al, 2011).

Polysaccharides are recovered from the fermentation broth of bacterial or fungal cultures.For sustainable and economical production of bioactive polysaccharides at industrial scale, instead of plants and algae, microbial sources are preferred as they enablefast and high yielding production under fully controlled fermentationconditions using economically cheap raw materials. Microbial production is achieved within days and weeks as opposed toplants where production time takes 6 or more months and also affected by environmental variations. Moreover, microbial production of EPS is also suitable for utilizing differentorganic resources as fermentation substrates (Donot et al, 2012).

Microorganisms used in industrial production of extracellular polysaccharides aremainly pathogenic bacteria. Species of *Xanthomonas*, *Leuconostoc*,*Pseudomonas,* and *Alcaligenes* which produce xanthan,dextran, gellan, and curdlan, respectively, are the mostwell known and most industrially used. Actually, the EPSsproduced by lactic acid bacteria (LAB), which are alreadyaccepted as GRAS (generally recognised as safe) representthe most suitable polymers for the food industry (Kranenburg et al, 1999).

Nevertheless, industry is constantly looking for EPS'swith novel functional properties to satisfy the needs of modern technology. Further, there is a pressingdemand for industry to resort to non-toxic, biodegradable,environmentally friendly substances.Extreme environments are proving to be a valuablesource of microorganisms that secrete interesting new biomolecules, including exopolysaccharides. Halophilic bacteria are just such extremophiles and the propertiesof their extracellular polysaccharides seem to offernumerous applications in various fields of industry(Margesin and Schinner 2001).

It is now demonstrated that the yields and compositions of the EPS were affected by different culturing conditions, particularly when using different carbon sources. However, no data are currently available concerning the effect of carbon sources on the change in chemical characterization and bioactivity of exopolysaccharide of archaealexopolysaccharide.

Recently, some studies revealed that the compositionsof the growth medium can affect the EPS synthesis. It has also been reported that themolecular size, degree of branching and constituents ofpure EPS depended on the growth medium(Hung et al, 2003).EPSs synthesis is generally favoredby presence of carbon source in excess, concomitant withlimitation by another nutrient (e.g., nitrogen, oxygen) (Nicolaus et

al, 2010).

Thus, our research has been aimed at establishingthe best carbon source for producing greater quantities of EPS for future biotechnological use from strain*Natrialba wudunaoensis SSBJUP-5.*

II. MATERIALS AND METHODS

Retrieval of haloalkaliphilic archaeal culture:–

The culture of*Natrialba wudunaoensis SSBJUP-5* was retrieved on Tyndall medium (Tindall et al, 1980) meant especially for haloalkaliphilic organism. It was freshlyinoculated on it and used for further study and plates were incubatedat 40^{o}C for 15 days.

Preparation of Inoculum:–

After fifteen days of incubation the biomass wasobtained from which inoculum was prepared. 5% of inoculum wasprepared in 14% saline water till the O. D. reached to 0.4 at 530nmwhere the cell density reached to 10^8 cells/ml as determined and adjusted by microscopic counting in Neubauer Chamber of the haemocytometer (Wilson, 2005).

Production of extracellular polysaccharide:-

Haloalkaliphilic growth medium (100ml) with 0.5%,0.75% and 1% carbon source in 250 ml capacity of conical flask wasinoculated with 5 ml of inoculum and broth was incubated at 400C for15 days. Different carbon sources such asglucose, lactose, sucrose, maltose and galactose are added instead of caseinhydrolysate in Tyndall medium with varying concentration 0.5%, 0.75% and1.0%. As a control original Tyndall broth containing casein hydrolysate was used.

Extraction of extracellular polysaccharide:-

The polysaccharide produced in the broth wasextracted by the ethanol precipitation method. The cells wereseparated from the polysaccharide in the broth by centrifugation at10,000 rpm for 15 min. Then, the pellet was decanted and supernatantwas precipitated with 2-3 volume of ice cold ethanol.Then, kept the flask in refrigerator for overnight.Polysaccharide forms precipitate at the bottom of the flask. Theprecipitate was separated by centrifugation at 6000 rpm for 15 min.The powder was transformed to preweighed dry petri dish and driedfor 24 hrs in the incubator at 400C.After 24 hrs, the difference between the dry weightsgave the amount of EPS in the fermented broth. Presence of crystalsin dried petri plate needs repeated centrifugation with d/w at 6000rpm for 10 min.

To Study the effect of different Carbon Sources

To study the effect of carbon sources glucose,sucrose, lactose, galactose and maltose sugars were used as a sourceof carbon with different concentration 0.5%, 0.75% and 1.0%. The dried powder was collected in another test-tube containing 5 ml D/W (warm). Standard glucose graph was plotted as per the phenol sulphuric acid method and amount of carbohydrate were estimated (Masuko et al., 2005).

III. RESULTS AND DISCUSSION

The focus of present study was to study effect of five different carbon sources with different concentrations on production of exopolysaccharide. The yield of the EPS produced was determined and expressed in terms of mass (gm/ml dry mass) as well as carbohydrate content in mg/ml.

Five carbon sources Glucose, Sucrose, Lactose, maltose and galactose in 0.5%, 0.75% and 1% concentration were used in this study. The control contained Casein hydrolysate as the sole carbon source. From results it is clear that, glucose was the most suitable carbon source for the production of EPS, having highest yield of 0.525% by mass (Table 4.1 and Figure 4.1) and 96 µg/ml by chemical estimation at 0.75% concentration of glucose in the medium (Table 4.2 and Figure 4.2).

Glucose was followed by sucrose that gave the highest yield at 1.0% concentration and lactose at 0.5% concentration in the medium. Galactose was the poorest carbon source that gave a poor yield of just 10 µg/mL at 0.75% concentration while 0.5 and 1.0% concentration did not favour the production of the EPS at all.Both glucose and fructose are hexoses, which can participate in glycolytic and pentose phosphate pathways and thus can be easily available for the growth and EPS production.

Table 4.1 Yield of EPS as dry mass (gm/mL) with different concentrations (%) of test carbohydrates.

Sr. No.	Carbon source	Yield of EPS (gm/mL dry mass) with test carbohydrate (%)		
		0.5	0.75	1
1	Glucose	0.2	0.525	0.02
2	Sucrose	0.112	0.026	0.226
3	Lactose	0.19	0.026	0.054
4	Maltose	0.088	0.1	0.092
5	Galactose	0	0.01	0
6	Control		0.203	

Fig. 4.1 Graphical representation of EPS yield (gm/ml dry mass) with different test carbohydrates.

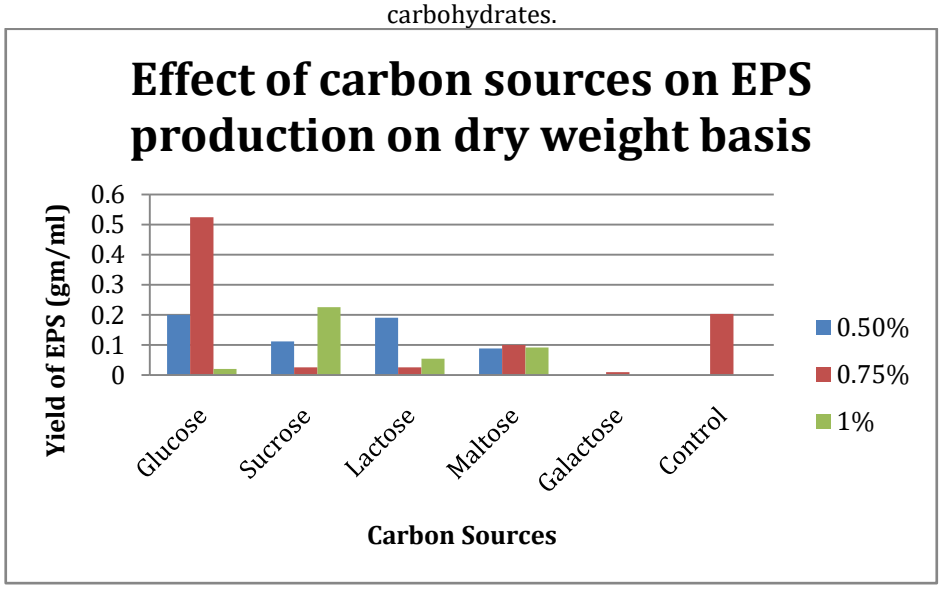

Table 4.2 Yield of EPS in terms of carbohydrate content with varying concentration (%) of test carbohydrates.

Sr. No.	Carbon Source	Yield of EPS (mg/mL) with varying concentrations (%) of test carbohydrates		
		0.5	0.75	1
1	Glucose	72	96	8
2	Sucrose	42	26	86
3	Lactose	74	24	40
4	Maltose	40	48	42
5	Galactose	0	10	0
6	Control	0	72	0

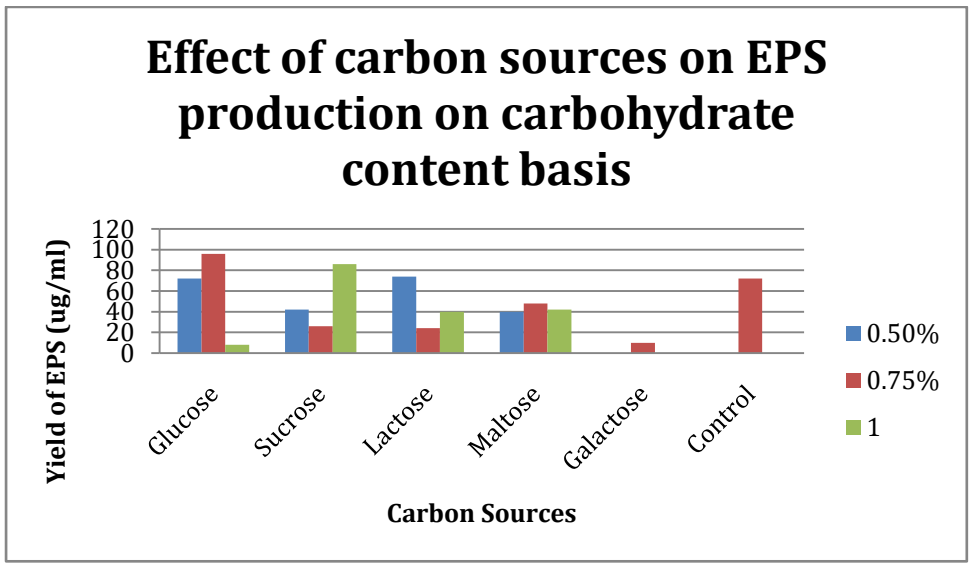

Fig. 4.2 Graphical representation of amount of carbohydrates (µg/ml) with different test carbohydrates

Similarly the results of the biomass and EPS of *P. vaninii* Ljup from the submerged culture with various carbon sources are showed that glucose and sucrose were the most suitable carbon source for biomass production. The highest EPS yield of 1.96 g/L achieved when sucrose was employed while in present study the yield obtained was 0.525g/L. The result indicates that*Natrialba wudunaoensis* SSBJUP-5 has the ability to use different carbon sources, all of which have varied degree of stimulatory effect on EPS production.

It has also been reported that different carbon sources had different effects on the production of secondary metabolism (Tang et al. 2008, Khondkar et al. 2002).Kai et al. (2003) found that different carbon sources in the media can influence the hetero-polysaccharides synthesis by *Pestalotiopsis microspora*. When glucose was used as the carbon source, a considerable amount of mannose units were formed. While with xylose as the carbon source, polysaccharides containing large amounts of mannose and galactose units were produced. Smiderle et al. (2012) also reported the similar results when glucose, galactose, xylose or arabinose was used as carbon source to produce polysaccharide by *Pleurotus pulmonarius*.

To our knowledge, EPS havenot so far been reported from Haloalkaliphilic archaea. The present study indicatesthat *Natrinema sp.*SSBJUP-1may be a potential source of EPS and may be of commercial value.

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POTASSIUM SOLUBILIZING BACTERIA – A BOON TO PLANT GROWTH

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ABSTRACT: *Potassium (K) is third most essential macronutrient after the Nitrogen (N) and Phosphorous. It is essential for growth and development of plants. Potassium plays its role in photosynthesis, protein synthesis and many metabolic pathways. Deficiency of potassium causes disease like chlorosis and its deficiency shows direct effect on the growth of plants. Abundant amount of potassium is present in soil but it is in insoluble rocks and minerals (mica, feldspar, illite) which cannot be easily accessible for plants. Potassium solubilizing bacteria are able to solubilize insoluble form of potassium into soluble form by secreting some acids, enzymes etc. which can be easily uptaken by plants. The excess use of chemical fertilizers affects soil fertility. So instead of using chemical fertilizers it is better to use potassium solubilizing bacteria which enhance the growth of plant by solubilizing insoluble K. This article presents an overview on Potassium Solubilizing Bacteria (KSB), their importance, mechanism and effect on plants growth rate.*

Keywords: *Potassium solubilizing bacteria, solubilization mechanisms, isolation methods, role of KSB in plant growth*

I. Introduction

Nutrients are essential for a plant to grow healthy. There are about 13 mineral nutrients in the soil and they are classified into two categories which are macronutrient and micronutrient depending on the quantity required (Lack and Evans, 2005). Among the Nitrogen (N), Phosphorus (P) and Potassium (K), Potassium is the third important plant macronutrient (Prajapati, 2012). Potassium (K) is seventh most common element in the earth's crust. It constitutes about 2.5 per cent of the lithosphere. However, actual soil concentrations of this nutrient vary widely ranging from 0.04-3.00 per cent (Sparks and Huang, 1985). Potassium plays significant roles in activation of several metabolic processes including protein synthesis, photosynthesis, enzymes, as well as in resistance to diseases and insects etc (Rehm and Schmitt, 2002). Potassium plays a important role in translocation of carbohydrates, water relations and sustains balance between monovalent and divalent cations (Brar and Tiwari, 2004). The major role of *potassium*is to provide the ionic environment for metabolic processes in the cytosol and as such functions as a regulator of various processes including growth regulation (Leigh and Wynj, 1984).

Potassium also functions in physiological processes such as activation of some enzymes, phloem solute transport of photoassimilates into source organs and maintenance of cation: anion balance in the cytosol and vacuole (Hopkins 2004). Potassium has also been implicated to have a role in the thickening of cell walls (Dantoff *et al*., 2007). Typical symptoms of potassium deficiency in plants include brown scorching and crushing of leaf tips as well as chlorosis (yellowish) between leaf veins. Purple spots may appear on the leaf undersides. Plants growth rate, root, seed and fruit development are usually reduced in potassium deficient plants. Often potassium deficiency symptoms first appear on older leaves because potassium is mobile nutrient. Meaning that a plant can allocate potassium to younger leaves when it is potassium deficient (Hopkins, 2004). Potassium though present in as abundant element in soil or is applied to fields as natural or synthetic fertilizers, only one to two per cent of this is available to plants, the rest being bound with other minerals and therefore unavailable to plants. The most common soil components of potassium, 90 to 98%, are feldspar and mica (McAfee, 2008).

Highest proportions of potassium in soils are in insoluble rocks and minerals (Goldstein, 1994) such as micas, illite, feldspar and orthoclases.The excess use of chemical fertilizers in agriculture are costly and have various adverse effects on soils that is reduces water holding capacity,soil fertility and disparity in soil nutrients (Goldstein, 1994). Soil microorganisms plays a central role in ion cycling, soil fertility and it also influence the availability of soil minerals (Bin Lian *et al*., 2010). The considerable populations of potassium solubilizing microorganisms are present in rhizospheric soils which promote the plant growth (Sperberg, 1958) and they can convert the insoluble or mineral structural potassium compounds into soluble forms in soil as a soil solution and make them available to the plants (Zeng *et al*., 2012).

Silicate bacteria were found to resolve potassium, aluminium andsilicon from insoluble minerals (Alexander, 1985). Certain bacteria are capable of decomposing alumina silicate minerals and releasing a portion of the potassium contained therein (Biswas and Basak, 2009). Potassium solubilizing bacteria (KSB)

are able to solubilize rock K mineral powder, such as micas,orthoclases andillite, through production and excretion of organic acids (Friedrich, 1991; Ullman, 1996). Organic acids can directly enhance dissolution of potassium minerals (Sugumaram and Janarthanam, 2007). Though several laboratory techniques states that bacteria produces bacterial acids, alkalis, acidic polysaccharides etc. the actual mechanism of potassium released from the minerals still not known (Groudev, 1987). Mineral potassium solubilisation by microorganisms which enhances crop growth and yield when applied with as cheaper source of rock potassium may be agronomically more useful and environmentally more feasible than soluble potassium (Rajan*et al*., 1996). Therefore, it is feasible method to increase growth and yield of plants by inoculating potassium solubilizing bacteria which solubilize insoluble potassium into soluble and makes potassium available for easy uptake in plants.The objective of this review article is to elaborate the studies of KSB including its ecology, importance of KSB in plant growth, laboratory isolation methods of KSB, mechanism of solubilizing insoluble K and effect of KSB on plants growth to develop efficient bacterial inoculants for solubilization of K in soil, which is one of the aim for sustainable development of agriculture.

Ecology of KSB

Soil is a complex mixture of minerals, water, air, organic matter, billions of organisms, and the changes taking place in its composition (biogeochemical transformations). Soil fertility refers to the capacity of the soil to supply essential plant nutrients such as N, P, K and micronutrients, which are often not available in free form or are in limited quantities in the soil. This is where root-associated beneficial microbes are important partners (de Zelicourt *et al*., 2013). The region of soil around plant roots known as the rhizosphere and up to 10 billion bacterial cells inhabit each gram of rhizospheric soil. In 2011, a team detected more than 33,000 bacterial and archaeal species on sugar beet roots(Jop de, 2015). Various research papers and books have revealed that soil management practices have also positive effect on soil microbial and faunal activities and increase soil microbial populations, their diversity and functions (Maurya *et al.,* 2014). It is possible to find various types of microorganisms from soil such as bacteria, fungi, actinomycetes, protozoa, and algae which bacteria are by far the most common (i.e., ~ 95%). There is an estimated 60,000 different type of bacteria that reside in the soil, most of which have yet to be even named, and each has its own particular roles and capabilities. The number and diversity of bacteria are influenced by the soil conditions such as organic carbon, temperature, moisture, electrical conductivity and other chemicals as well as by the number and types of plants found in those soils. Therefore, soil-grown plants are immersed in a sea of microorganisms especially bacteria (Glick, 2012). Furthermore, symbiotic nitrogen fixing rhizobia and *Pseudomonas*have also shown K and P-solubilizing activity, which fix atmospheric nitrogen into ammonia and export the fixed nitrogen to the host plants (Uroz *et al* 2009).KSB are also known as plant growth promoting bacteria (PGPRs) (Gundala *et al*., 2013).

Importance of potassium solubilizing bacteria (KSB) in plant growth

The concentration of soil solution K varies from 2 to 5 mg l^{-1} for normal agricultural soils (Sparks and Huang, 1985).Deficiency of K has become important limiting factors for the development of agriculture. Indiscriminate use of synthetic fertilizers has led to pollute water basins, contamination of soil,destroyed microorganisms and friendly insects,reduced soil fertility and making the crop prone to diseases (Doman and Geiger, 1979).The KSB also produce amino acids, vitamins and growth promoting substances. One possible way to mitigate K deficiency is by the use of indigenous efficient potassium solubilizing microorganisms (KSMs) that play key roles in K-solubilization through different mechanisms like acidolysis, chelation, exchange reactions, complexolysis and production of organic acids (Archana *et al*., 2012).In addition to increasing plant resistance to diseases, pests, and abiotic stresses, K is required to activate over 80 different enzymes responsible for plant and animal processes. e.g. such as energy metabolism, starch synthesis, nitrate reduction, photosynthesis, and sugar degradation (Etesami*et al*., 2017).Now certain species of microorganisms like potassium solubilizing microorganisms (KSM) are widely used as a good substitute of chemical fertilizer. Besides above fact, the long term use of bio fertilizers is eco-friendly, more efficient, productive and accessible to marginal and small farmers over chemical fertilizers (Reyes *et al*., 2001). A biofertilizer containing KSB, it not only improves fertility of soil but also increases yield of crops, protects from harmful diseases and reduce application of other chemical fertilizers (Sheng *et al*., 2003, Sindhu *et al*., 2010). After the finding of many researchers, it shows that the potassium solubilizing bacteria is important for the growth of plants which is effective in solubilizing insoluble potassium into soluble form.

Laboratory isolation of KSB

KSB are usually present in all soils and have been isolated fromnon-rhizosphere soil,rhizosphere soil, paddy soil (Bakhshandeh *etal*., 2017) and saline soil (Bhattacharya *et al*., 2016).Sugumaran and Janartham(2007) collected soil samples and were mixed with insoluble potassium (Feldspar) and incubated for 1 week at room temperature. After adaptation 1 gm of soil was inoculated in 100 ml liquid medium containing 1% glucose, 0.05% yeast extract, 0.5% feldspar and incubated at 37º C on 120 rpm for 1 week. Enriched samples were inoculated after serial dilution up to 10^{-6} on Aleksandrov agar medium constituted 1% glucose, 0.05% $MgSO_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPO_4$ and 0.5% potassium aluminium silicate, agar 3 % pH-6.5 and incubated at 37º C for 1 week.Aleksandrov medium is a selective medium for the isolation of potassium solubilizers, containing insoluble potassium bearing mineral (mica) (Parmar *et al*., 2016).

One gram of rhizosphere soil was mixed thoroughly in 100 ml sterile water and wasprocessed following serial dilution agar plate technique. Suitable dilutions (10^{-5} and 10^{-6}) of both rhizosphere and rhizoplane solutions were plated on Aleksandrov medium(Hu *et al.*, 2006).

KSB were isolated by serial dilution plate method using modified Aleksandrov medium. The pH of this medium was adjusted to 7.2 by adding 1 N NaOH. The plates were incubated at 28 ± 2 °C in biological oxygen demand incubator for 3-4 days. The colonies exhibiting clear zones were selected and diameter of the solubilization zone was calculated in mm (Etesami*et al.,* 2017).

KSB were cultured in Tryptone Yeast medium (Vincent, 1970) and sucrose-minimal salts medium (Sheng *et al*., 2002) respectively, and incubated on an orbital shaker at 150 rpm for 48 hr at 27°C.Zhang and Kong (2014) isolated potassium solubilizing bacteria from rhizospheric soil on Aleksandrov selective medium.

Recently, Rajawat *et al.,*(2016) suggested a modified plate assay for rapid screening of KSB. This assay was based on improved visualization of halo zone formation around the colonies on agar plates, through inclusion of an acid-base indicator dye (bromothymol blue), to modify the Aleksandrov medium. This assay is also time-saving, more sensitive, and beneficialin comparison to the Aleksandrov plate assay.

On the basis of literature review it was observed that Aleksandrov media is suitable for the growth of KSB which contains mica as a source of insoluble form of potassium and all essential nutrients.

Role of KSB in solubilisation of minerals

Potassium is present in soil either in the complexed form or chelated insoluble mineral form in mica or illite, this is dissolved by potassium solubilizing bacteria by releasing organic acid to convert complex form into simple form and make K available to plant (Aleksandrov, 1967; Bennett, 1998).The production of organic acids such as acetate, citrate and oxalate by microorganisms can increase mineral dissolution rate (Hazen *et al.*, 1991 and Barker *et al.*, 1998). Moreover results also suggest that the weathering ability of the bacteria which involves the production of protons, organic acids, siderophores and organic ligands (Grayston *et al.*, 1996; Liermann *et al.*, 2000; Paul and Clark, 1989; Rogers *et al.*, 2004; Welch *et al.*, 1999).

Freidrich *et al.,*(1991) and Ullman *et al.,*(1996) reported (KSB) potassium soluiblizing bacteria *B. mucilaginosus* are able to soluiblize rock K mineral powder such as micas, illite and orthoclases through production and excretion of organic acids.

These potassium solubilizing bacteria (KSB) were found to dissolve potassium, aluminiumand silicon from insoluble K-bearing minerals such as micas, orthoclases and illite by excreting organic acids which either directly dissolved rock K or chelated silicon ions to bring K into the solution (Aleksandrov, 1967; Ullman, 1996; Bennett, 1998).

In general, the most important mechanisms known in K mineral solubilization by KSB are "(i) by enhancing chelation of the cations bound to K; (ii)by lowering the pH; and (iii) acidolysis of the surrounding area of microorganisms (Meena *et al*., 2014).

Palmer *et al.,*(1991), Welch *et al.,*(1993) and Styriakova *et al.,*(2003) reported organiccompounds produced by micro-organisms such as acetate, citrate and oxalate can increasemineral dissolution rates in laboratory experiments and in the soil by Sheng *et al.,*(2003),Badr *et al.,*(2006).

The major mechanism of K mineral solubilisation is by production the organic, inorganic acids and production of protons (acidolysis mechanism) which are able to convert the insoluble K (mica, muscovite, and biotite feldspar) to soluble forms of K, easily taking up by the plant (Etesami*et al*., 2017). Sheng and He (2006) reported that solubilization of insoluble K by microorganisms is due to the production of organic acids like oxalic acid and tartaric acids and also due to production of capsular polysaccharides which helps in dissolution of minerals to release potassium. Decomposition of silicate minerals by *B. mucilaginosus* due

to production of oxalate and citrate and the extent of which polysaccharides absorbed organic acids decomposes minerals (Liu *et al.*, 2006).

Increasing evidence also exists for a mechanism of direct silicate precipitation bybacteria via metal sorption at the cell membrane (Archana, 2007).The types of various organic acids such as oxalic acid, tartaric acids, gluconic acid, 2-ketogluconic acid, citric acid, malic acid, succinic acid, lactic acid, propionic acid, glycolic acid, malonic acid, fumaric acid, etc. have been reported in KSB, which are effectivein releasing K from K-bearing minerals (Etesami*et al*., 2017).

In addition to decreasing soil pH, organic acids produced by KSB can release of K ions from the mineral K by chelating (complex formation) Si^{4+}, Al^{3+}, Fe^{2+}, and Ca^{2+}ions associated with K minerals (Meena *et al*., 2014; Römheld and Kirkby, 2010; Štyriaková *et al*., 2003).

The *B. altitudinis* strain could accelerate weathering of potash feldspar, change mineral surface morphology, and induce the formation of new mineral complex. This strain dissolved potash feldspar and significantly released more Si, Fe, and Al elements by producing organic acids (Huang *et al*., 2013).It was found oxalic acid production caused dissolution of fledspar while oxalic and tartric acid were involved in mobilizing illite (Hu *et al*., 2006).Some bacteria like *Bacillus*, *Thiobacillus,Pseudomonas*, *Acidothiobacillus*has been found to simplify and secrete potassium from potassium-bearing complex minerals in soils (Sheng, 2005; Liu, 2012).The mechanism of potassium solubilization means by which insoluble potassium and structural unavailable forms of potassium compounds are solubilized due to the production of various type organic acids such as *Pseudomonas trivalis*produces gluconic, lactic, succinic, formic and malic acid.*Serratia marcescens*produces citric and lactic acid as well as *Enterobacter sp.*produces malic and gluconic acid (Reyes, 2001).

It has been reported that the production of various extracellular polymers (primarily proteins and polysaccharides) can also be led to release of K from K bearing minerals for plant uptake (Liu *et al*., 2006; Shelobolina *et al*., 2012; Sheng and He, 2006).Another report by Groudev (1987) showed that potassium was soluiblized by production of inorganic and organic acids and due to production of mucilaginous capsules containing of exopolysaccharides by *Bacillus, Clostridium* and *Thiobacillus.*

Solubilization of K by KSB from insoluble and fixed forms is an import aspect regarding K availability in soils. The ability to solubilize the silicate rocks by*A. ferrooxidans, Arthrobacter* sp.,*B. mucilaginosus, B. circulanscan, B. edaphicus*, *Burkholderia, Enterobacter hormaechei, Paenibacillus mucilaginosus, P. frequentans, Cladosporium, Aminobacter, Sphingomonas,* and *Paenibacillus glucanolyticus* has been reported (Meena *et al*., 2016). Amongthe soil bacterial communities, *B. mucilaginosus, B. edaphicus and B. circulanscan* have been described aseffective K solubilizers (Meena *et al.*, 2014; Meena *et al*., 2015a; Meena *et al*., 2016).

Welch and Vandevivere (1994) suggested KSB also synthesizes biofilms, which create controllable microenvironmentsaround microbial cells for weathering (Meena*et al.*, 2014).In addition, it is known that the release of organic acids from the plant roots can be effective in enhancing mobilization of mineral K (Wang *et al.*, 2000).

Among the different organic acids involved in the solubilisation of insoluble K, tartaric acid, citric acid, succinic acid, α-ketogluconic acid, and oxalic acid are the most prominent acids released by KSB (Meena *et al.*, 2014).

Effect of KSB on plant growth

The soils are getting exhausted in K stock at a faster rate because of introduction of high yielding crop varieties and theintensification of agriculture (Ethseami *et al*., 2017). The inoculations with KSB and other useful microbial inoculants in the soil become mandatory to restore and maintain the effective microbial populations for solubilization of chemically fixed potassium and availability of other micro and macronutrients to harvest good sustainable yield of various crops(Maliha *et al.,*2004).

The increase in yield of cotton showed by 50-94 per cent when *Azotobacterin* and silica bacterin were applied simultaneously (Ciobanu, 1961).Inoculation of seeds and seedlings of different plants with KSB generally showed significantenhancement in plant growth, yield, germination percentage, seedling vigor, and K uptake by plants under greenhouse and field conditions (Anjanadevi *et al*., 2016; Awasthi *et al*., 2011; Lynn *et al*., 2013; Meena *etal*., 2015a; Meena *et al*., 2014; Subhashini and Kumar, 2014; Zhang *et al*., 2013; Zhang and Kong, 2014).

Badr *et al*., (2006) reported that application of KSB with K- and P-bearing minerals on sorghum enhanced dry matter yield by 48 %, 65 %, and 58 %; phosphorus uptake by 71 %, 110 %, and 116 %; and K

uptake by 41 %, 93 %, and 79 % in clay, sandy, and calcareous soils, respectively.

Xue *et al.,*(2000) and Sheng *et al.* (2003) reported silicate dissolving bacteria could improves soil P, K, Si reserves and promote plant growth. The effect of potash mobilizer on brinjal has recorded an increased potash uptake and increased plant biomass in potash mobilizer treated plants as compared to the control plants (Nayak, 2001).Co-inoculation of KSB and PSB in conjunction with direct the application of rock K and Pmaterials into the soil increased N, P and K uptake,photosynthesis and the yield of eggplant grown on P and K limited soils (Han and Lee, 2005).

Nowadays, biofertilizer is an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. Furthermore, the use of biofertilizer has gained momentum in recent years since chemical fertilizers are high cost and can cause hazardous effect (Aseri *et al*., 2008).By using potash solubilizer in combination with other biofertilizer like *Rhizobium, Azospirillum, Azotobacter, Acetobacter* and PSM showed increase in yield by 15 to 20 per cent in yam and tapioca (Chandra *et al.* 2005). By identifying efficient bacterial strains which are capable to solubilize K minerals can quickly conserve our existing resources and avoid environmentalhazardous pollution caused by heavy application of K-fertilizers (Etseami *et al*., 2017).

Limitations for use of KSB as biofertilizers(Etseami *et al*., 2017)

1. Soil limitation or constraint
   Due soil and climatic conditions, high fertility status, high temperature, unfavourable pH, drought, deficiency of potassium containing mineral(mica, illite, orthoclases) and other essential minerals or toxic elements affect the microbial growth and crop response.
2. Commercial production limitations
   Due to deficiency in technology with respect to inappropriate product formulation of KSB as biofertilizer.
3. Unavailability of appropriate and efficient strains
   Lack of region specific potassium solubilizing bacterial strains is one of the major limitations for production of KSB. As potassic biofertilizers are not only crop specific but also soil specific. Another reason for unavailability of strains is culture collection centers are not yet developed for KSB due to lack of efficient strains developed by scientists.
4. Market level limitations
   Lack of awareness - majority of farmers are not aware about KSB-biofertilizer, their uses in increasing the quality and yield of crops.
5. KSB resource limitations
   The investment in production of KSB-biofertilizer unit is very low, there is limited resources are available for production of KSB-biofertilizer.

II. CONCLUSION

Various research outcomes on presence of soil microorganisms were revealed its positive effect on enhancing growth of various crops. Microbial population including bioinoculants, biofertilizers, NP solubilizing bacteria, mycorrhizae, actinomycetes, protozoa etc. were proved to have ability to increase soil fertility and enhance crop yield. In addition, use of chemical fertilizer also showed adverse effect on soil microbial population, soil fertility and ecosystem. Besides this facts use of KSB is best substitute to chemical fertilizer to improve crop fertility which solubilizes insoluble potassium into soluble form. It also increases organic acid production, create ionic environment into plant vicinity. All of these aspects showed beneficial effect on plant growth.

In the view of above context, it is necessary to increase commercial production, availability, market potential and awareness among the farmers about use of KSB as a biofertilizer to increase crop yield.

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APPLICATION OF QUALITY BY DESIGN (QBD) APPROACH IN DEGRADATION STUDY OF SIBUTRAMINE

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ABSTRACT: *In normal Forced degradation study the drug is subjected to extreme chemical and environmental conditions to determine product breakdown levels and preliminary degradation kinetics, and potential degradation products. As novelty in work we have applied QbD approach whereby critical quality attributes related to various process of degradation like hydrolytic, photolytic, thermal and oxidative are identified and optimized. Due to application of QbD approach for analytical method development along with validation additional accuracy and precision is seen in the final results.*

In present work a simple, precise, accurate and stability indicating UV-method have been developed and validated for estimation of Sibutramine. For analysis of Sibutramine absorbance maxima was found to be 239 nm. And Beer's range was found to 2-16µg/ml. For stability study conditions were optimized in such a way that degradation between 5-20% of Sibutramine can be achieved as per the ICH requirement.

Using QbD approach in degradation study for acid degradation 1N HCL, for alkali degradation 0.1 N sodium hydroxide, for oxidative degradation 0.1% hydrogen peroxide, for thermal degradation at 60°c temperature, for photolytic degradation long wavelength were used.

Keywords: *Sibutramine, Beer's law, Analytical method validation, Forced degradation, QbD.*

I. INTRODUCTION

Forced degradation (FD) study is a process by which the natural degradation rate of a pharmaceutical product is artificially increased by the application additional stressful conditions. [1] FD studies help in identify reactions that may be responsible for causing degradation of pharmaceutical product. They are being used as part of the development strategy and an integral component of validating analytical methods that indicate stability and detect impurities which are formed during manufacture, storage, or use and their properties are different from the desired product with respect to activity, efficacy and safety. The analytical methods are developed for determine the degradation products formed during accelerated and long term stability studies. Degradent may be hazardous, as they may cause toxic effects like cytotoxicity, genotoxicity or carcinogenicity. Hence regulatory authorities are insisting on development of stability related analytical profile.

QbD is "a systematic approach to development that begins with predefined objectives and emphasizes understanding and control, based on sound science and quality risk management" The outcome of using QbD concepts is a well-understood product and process that consistently delivers its intended performance. In present work the QbD approach has been applied to analytical methods used in force degradation study.

QbD approach has been applied to analytical method like the UV method as well as methods used for force degradation for which conditions like hydrolytic, thermal, photolytic and oxidative are applied. [2] In all these methods identification of critical quality attributes, which can affect the end results are identified.

For example, in hydrolytic degradation study critical quality attributes will be strength of acid as well as alkali, its quantity and time for which exposure is done. If all these parameters are optimized as per analytical guidelines the generation of specific amount of degradation product is possible and along with validation of method would provide more amount of accuracy to the results of analytical method. [3]

II. MATERIAL AND METHOD

2.1 INSTRUMENTS

A shimadzu UV-visible spectrophotometer (UV 1800 shimadzu) connected to computer loaded with spectra management software, 10 mm matched quartz cells were used for all the absorbance measurement.

2.2 MATERIAL

Sibutramine was obtained as gift sample from stride pharmaceuticals, Bangalore, India. All

chemicals like HCL, methanol, sodium hydroxide etc. were of analytical grade.

2.3 MOBILE PHASE

Methanol (100%) was used as mobile phase.

2.4 STOCK SOLUTION

Standard stock solution of sibutramine (100µg/ml) was prepared and from this required concentration were used for analysis.

DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORPTION

For selection of analytical wavelength, 8µg/ml solution of sibutramine was prepared by appropriate dilution of standard stock solution and scanned in the spectrum from 400 to 200 nm using diluent as blank. From the spectra of drug. λ_{max} of sibutramine, 239nm was selected for the analysis. (Fig. 1)

2.6 PREPARATION OF WORKING STANDARD SOLUTIONS

The Prepared stock solution was further diluted with methanol to get working standard solution

2.7 PREPARATION OF CALIBRATION CURVE

It was observed that Beer's law is followed in concentration of 2 to 16µg/ml. (Table 1)

VALIDATION OF DEVELOPED METHOD

2.8 LINEARITY AND RANGE

For linearity study, different concentration solutions ranging from 2 to 16µg/ml were prepared by dilution of standard stock solution having concentration of different aliquots of stock solution, calibration plot is represented in (Fig. 2)

2.9 ACCURACY

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100%, and 120%) of the bulk sample of sibutramine.[3] (Table 2)

2.10 PRECISION

These precision of the proposed method was done by actual determination of three replicates of fixed concentration of the drug and finding out the absorbance by taking a fixed concentration of 8µg/ml by the proposed method. From these absorbance mean, Standard deviation, %R.S. D was calculated. Intra Day Assay The assay procedure was carried out in the same day in the duration of 2 hours to 3 hours at fixed concentration and the results were compared. Inter Day Assay procedure was carried out for single day with freshly prepared solution from stock solution at fixed concentration at 24hour interval. [3] (Table 3 and 4)

2.11 ROBUSTNESS

This procedure was carried out by changing the analyst within the same laboratory and same instrument. Then results were compared. [3] (Table 5)

FORCE DEGRADATION STUDY

2.12 ACID DEGRADATION

First in a 100ml volumetric flask, accurately weighed 0.8mg of bulk drug was dissolved with methanol, as application of QbD approach the strength, volume and time were identified as critical quality attributes. It was observed that o.5 ml of 1N HCL and half hour time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines [4] (Table 6 and Fig. 3, 4)

2.13 ALKALI DEGRADATION

First in a 100ml volumetric flask, accurately weighed 0.8mg of bulk drug was dissolved with methanol, as application of QbD approach the strength, volume and time were identified as critical quality attributes. It was observed that o.5 ml of 0.1N sodium hydroxide and half hour time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines [2, 5] (Table 7 and Fig. 5, 6)

2.14 PHOTOLYTIC DEGRADATION

First in a 2 different 100ml volumetric flask, accurately weighed 0.8mg of bulk drug was dissolved with few drops of methanol and volume was made by methanol in each volumetric flask respectively. Then first sample was kept at normal wavelength for half hour and second sample was kept at long wavelength for half an hour in UV cabinet. As application of QbD approach the range of wavelength, time were identified as critical quality attributes .and half hour time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines.[2,5] (Table 8 and Fig. 7, 8)

2.15 THERMAL DEGRADATION

First in a 2 different 100ml volumetric flask, accurately weighed 0.8mg of bulk drug was dissolved

with few drops of methanol and volume was made by methanol in each volumetric flask respectively. Then second sample was kept in hot air oven at 60°C for 2 hour and first sample was kept at room temperature for 2 hours. As application of QbD approach the temperature, time were identified as critical quality attributes .and 2 hour time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines [2, 5] (Table 9 and Fig. 9, 10)

2.16 OXIDATION WITH H_2O_2

In a 2 different 10ml volumetric flask, accurately weighed 1mg of bulk drug was dissolved with few drops of methanol and volume was made by methanol in each volumetric flask respectively. As application of QbD approach the strength, volume and time were identified as critical quality attributes. It was observed that 2 ml of 0.1% hydrogen peroxide and 12 minutes time interval was found to be critical for getting degradation in the range of 5-20% as per ICH guidelines [2, 5] (Table 10 and Fig. 11,

III. RESULT AND DISCUSSION

Fig 1: Typical UV Chromatogram Showing Sibutramine at 239nm

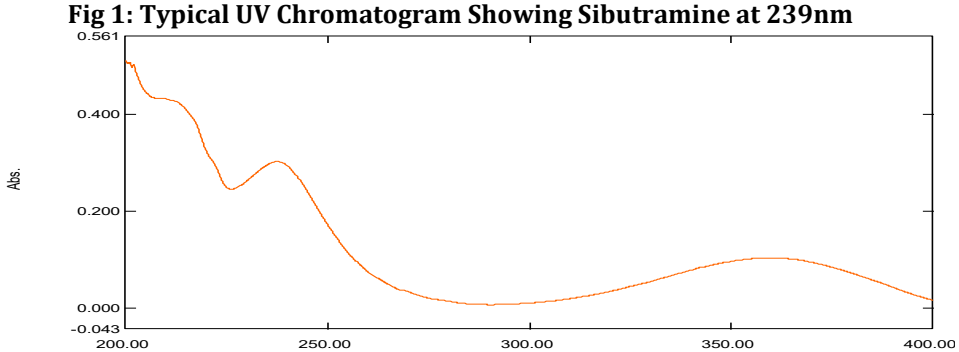

Table 1: Calibration range of sibutramine

CONCENTRATION (µ/ml)	ABSORBANCE (239 nm)
2	0.082
4	0.163
6	0.239
8	0.300
10	0.377
12	0.451
14	0.523
16	0.619

Fig 2: Linearity graph of sibutramine

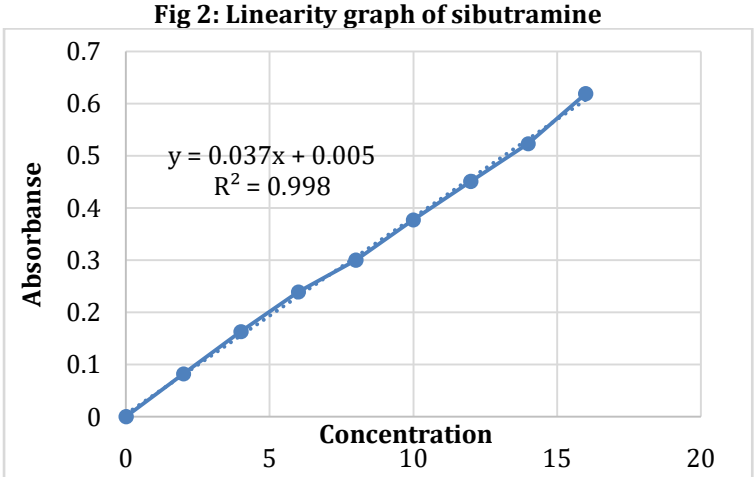

Table 2: Accuracy reading of sibutramine

SR.NO	% DRUG SOLUTION	% DRUG CONCENTRATION			% RSD
1	80	67.5	68,12	68.75	0.009175
2	100	83.12	82.5	85	0.015583
3	120	102.5	101.25	101.5	0.006301

Table 3: Precision day 1 reading of sibutramine

SR.NO	TIME	% DRUG CONCENTRATION			% RSD
1	MORNING	81.2	80	77.5	0.029432
2	AFTERNOON	85	85.5	86.25	0.007351
3	EVENING	90	88.75	92.5	0.021118

Table 4: Precision day 2 reading of sibutramine

SR.NO	TIME	% DRUG CONCENTRATION			% RSD
1	MORNING	83.75	77.5	75	0.057231
2	AFTERNOON	88.75	86.25	88	0.014634
3	EVENING	101.25	103.75	102.25	0.012286

Table 5: Robustness reading of sibutramine

SR.NO	ANALYST	% DRUG CONCENTRATION			% RSD
1	1	87.5	86.25	86	0.009282
2	2	83.75	86.25	80	0.037749

Table 6: Acid Degradation study of sibutramine

SR.NO	CONC. µg/ml	NORMALITY (N) OF HCL	VOLUME OF HCL ADDED (ml)	% DEGRADATION
1	8	0.01	0.5	26
			1	46
			1.5	47
2		0.1	0.5	4
			1	21
			1.5	50
3		**1**	**0.5**	**20**
			1	35
			1.5	45

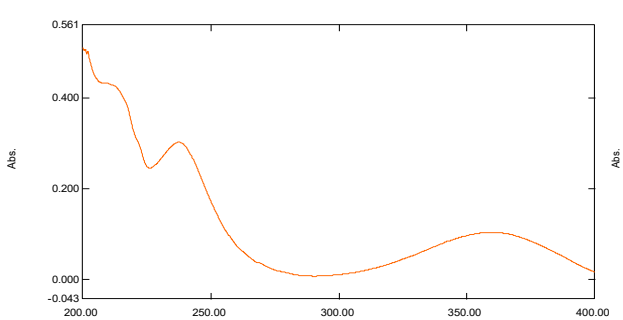

Fig 3: Spectra of sibutramine before acid degradation

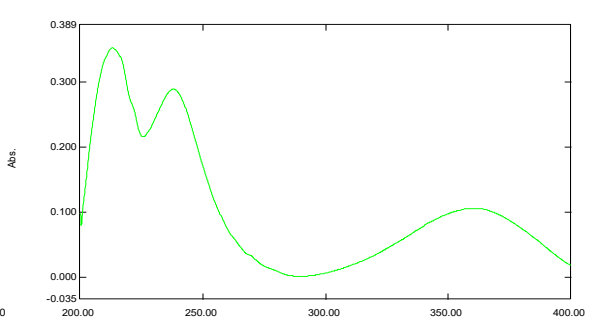

Fig 4: Spectra of sibutramine after acid

Table 7: Alkali degradation study of sibutramine

SR.NO	CONC. µg/ml	NORMALITY (N) OF NaOH	VOLUME OF NaOH ADDED (ml)	% DEGRADATION
1	8	0.01	0.5	28
			1	46
			1.5	54
2		0.1	0.5	11
			1	31
			1.5	41
3		1	0.5	21
			1	42
			1.5	45

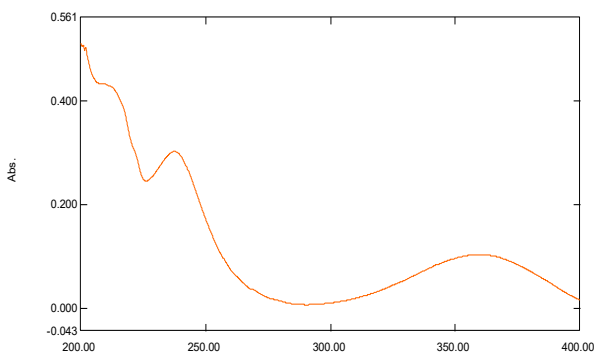

Fig 5: Spectra of sibutramine before alkali degradation

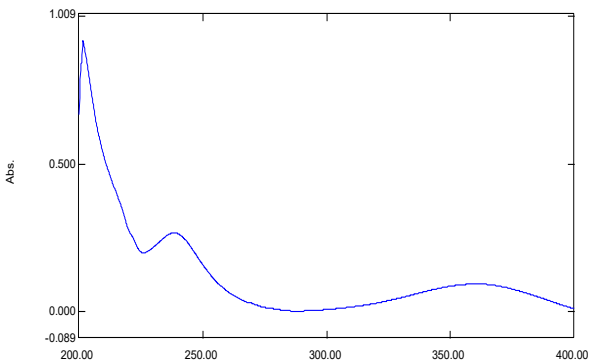

Fig 6: Spectra of sibutramine after alkali degradation

Table 8: Photolytic degradation of sibutramine

SR.NO	CONC. µg/ml	ABS. AT NORMAL WAVELENGTH	ABS. AT LONG WAVELENGTH	% DEGRADATION
1	8	0.173	0.162	7

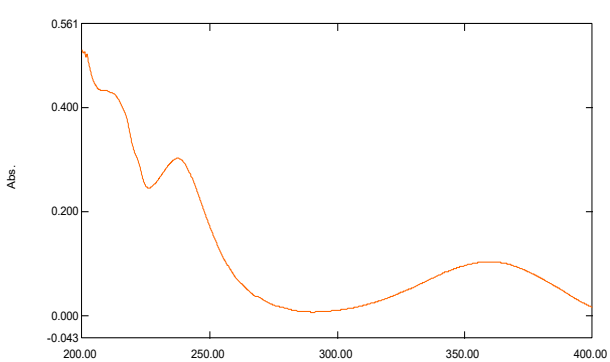

Fig 7: Spectra of sibutramine before photolytic degradation

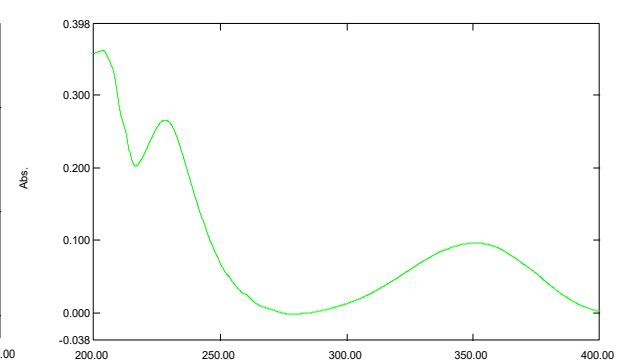

Fig 8: Spectra of sibutramine after photolytic degradation

Table 9: Thermal degradation of sibutramine

SR.NO	CONC. µg/ml	ABS. AT ROOM TEMPERATURE	ABS. AT TEMPERATURE (60°C)	% DEGRADATION
1	8	0.180	0.165	10

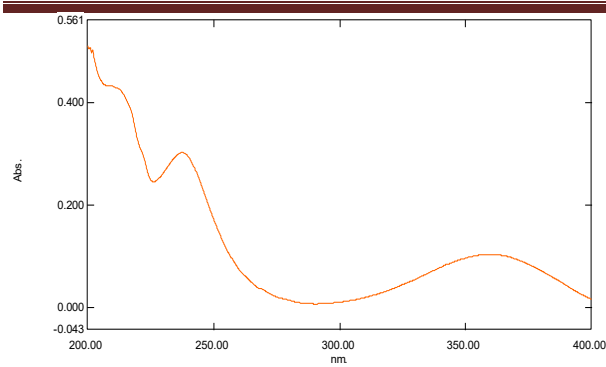

Fig 9: Spectra of sibutramine sample 1 photolytic degradation

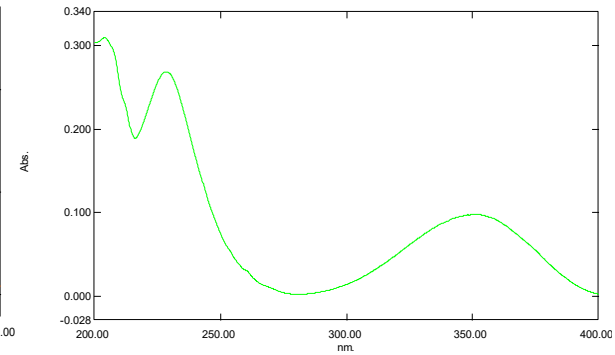

Fig 10: Spectra of sibutramine sample 2 photolytic degradation

Table 10: Oxidative degradation of sibutramine

SR.NO	CONC. µg/ml	% OF H_2O_2 ADDED	VOLUME OF H_2O_2 ADDED (ml)	% DEGRADATION
1	100	0.1	2	10
2	100	1	2	41

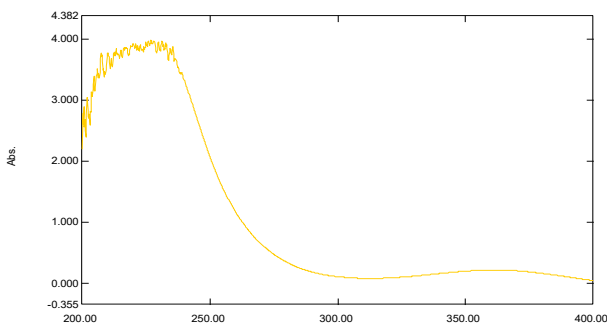

Fig 11: Spectra of sibutramine before oxidative degradation

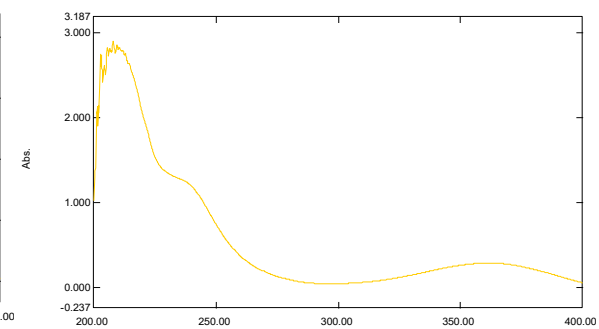

Fig 12: Spectra of sibutramine after oxidative degradation

III. CONCLUSION

From this we conclude that the method developed and validated was linear, calibrated, accurate, precise, robust, and reproducible. From UV- spectroscopy we conclude that UV method was suitable for the degradation study.

From degradation study we conclude that sibutramine was degraded between 5-20% as per ICH guideline and it shown degradation at acid, alkali, photolytic, thermal and oxidative.

IV. ACKNOWLEDGEMENT

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GCMS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *ACACIA NILOTICA* L. AGAINST *XANTHOMONAS AXONOPODIS* PV. *PUNICAE*

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ABSTRACT: *The present investigation was undertaken to evaluate the potential of Acacia nilotica L. as antibacterial agent against Xanthomonas axonopodis pv. punicae, phytopathovar of bacterial blight of Pomegranate (Punica granatum).The aqueous and solvent bark and seed extract of Acacia nilotica L. were evaluated by agar well diffusion method. The bark and seed extracts were prepared in six solvents viz. water, ethanol, methanol, diethyl ether, benzene, chloroform and tested for its antibacterial activity against Xanthomonas axonopodis pv. punicae. Aqueous bark and methanol seed extract of Acacia nilotica L. showed significant antibacterial activity against Xanthomonas axonopodis punicae. Minimum inhibitory concentration of aqueous bark and methanol seed extract of Acacia nilotica L. were 150 and 500µg/ml respectively. GCMS analysis of these extract were studied for presence of phytoconstituents that may have capability to inhibit the growth of Xanthomonas axonopodis punicae indicating their potential use in controlling bacterial blight of pomegranate.*

Keywords: *Acacia nilotica L. Xanthomonas axonopodis pv. punicae, antibacterial activity, Minimum Inhibitory Concentration, GCMS analysis*

I. INTRODUCTION

The pomegranate (*Punica granatum*) having high nutritional, economic and therapeutic value (Julie Jurenka, 2008). India is one of the major pomegranate producer country in the world with average production of 8 lakh tones per annum (Pawar *et al.*,2013). Presently yield and quality of Pomegranate is affected by bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae.* The pathogen can infect leaves, stems and fruits which develop black, water soaked oily lesions in early stage of infection which later become completely black, split and dry off. Further tissue necrosis occurs on leaves and twigs. The disease management includes use of chemicals such as Bordeaux mixture and antibiotics particularly Streptocycline. (Yenjerappa *et al.*, 2009). Use of chemicals in agriculture causes several adverse and environmental hazards (Shanthi, 2011). Hence there is growing interest worldwide in the utilization of eco-friendly materials for controlling the growth of the pathogen (Madhiazhagan *et al.*, 2002). Present study was undertaken to evaluate antibacterial potential of *Acacia nilotica* L. against *Xanthomonas axonopodis* pv. *punicae*, phytopathovar of bacterial blight of pomegranate (*Punica granatum*).

Acacia nilotica (L.) belongs to family Fabaceae (Malviya *et al.*, 2011). It is found commonly along roadsides, field bunds in open grasslands, near streams in India (Gore, 2015; Gaikwad and Gore, 2015). Raghavendra *et al*., (2006) reported that the aqueous, methanol and ethanol extracts of *Acacia nilotica* had significant antibacterial activity against all the pathovars of *Xanthomonas* and 14 human pathogenic bacteria. Mahesh and Satish(2008) reported antibacterial activity of methanol leaf extracts of *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifer* and *Ziziphus mauritiana* against *Bacillus subtilis, Escherichia coli*, *Pseudomonas fluorescens, Staphylococcus aureus* and *Xanthomonas axonopodis* pv. *malvacearum* and antifungal activity against *Aspergillus flavus, Dreschlera turcica* and *Fusarium verticillioides.* Fatima *et al.,* (2012) reported leaf, bark and root extracts of *Acacia nilotica* (L.) possessed antibacterial potential against *Xanthomonas malvacearum* by using agar well diffusion method and reported that the ethyl acetate extracts of root had antibacterial potential against *Xanthomonas malvacearum.* Taking into consideration these reports, the present study was undertaken to study the potential of aqueous and different solvent extracts of *Acacia nilotica* against *Xanthomonas axonopodis* pv. *punicae*.

II. MATERIALS AND METHODOLOGY

Isolation and identification of pathogen

Diseased Pomegranate fruits were collected from field located at village Kurul, district Solapur, Maharashtra, India. The infected portion of fruits was removed, and then surface sterilized with 0.1% mercuric chloride solution for one minute and washed three times with sterile distilled water. It was cut

with sterile scalpel and squeezed gently in sterile saline. The presence bacteria in fruit lesion were confirmed by performing ooze test. The suspension was serially diluted and plated on sterile Nutrient Glucose Agar medium (Beef extract-0.3%, peptone-0.5%, glucose-0.25%, agar-2%, pH-6.8) (Mondal *et al*., 2012) and incubated at 30ºC for 72 hrs. After incubation typical mucoid, yellow colored, well isolated colony was selected and studied for morphological and biochemical characteristics. The culture was further identified on the basis of Pathogenicity test. The isolate that showed symptoms of bacterial blight on leaves of pomegranate plant after performing pathogenicity test was subjected for molecular identification on the basis of 16s rRNA sequencing. The sequence in this study has been deposited in Gene bank data bases under accession number KP168824 (Gargade and Kadam, 2015).

Collection of leaves and seeds

Bark and seeds of *Acacia nilotica* (L.) were collected and identified from Department of Botany, Dayanand College of Arts and Science, Solapur, Maharashtra. India. The plant parts were washed thoroughly with running tap water and once with sterile distilled water. The plant materials were dried with blotting paper under shade.

Preparation of aqueous extract

Twenty five grams of dried bark and seeds of *Acacia nilotica* (L.) were macerated with sterile distilled water in warring blender separately. The macerate was kept for 24 hr at room temperature(Nidaullah *et al.*,2010).The macerate was filtered through double layered muslin cloth and was centrifuged at 4000rpm to separate supernatant .The filtrate was then stored at 4ºC.

Preparation of solvent extracts

Twenty five grams of dried bark and seeds of *Acacia nilotica* (L.) were filled in the thimble prepared with muslin cloth and extraction was carried out in Soxhlet apparatus by using different solvents. It was then concentrated by using rotary flash evaporator and preserved at 4ºC for further use (Raghavendra *et al.,* 2006). All the extracts were used to test antibacterial activity.

Antibacterial activity

Antibacterial activity of various extracts was studied by using agar well diffusion method. Cell density was adjusted to 10^6 -10^7 cfu/ml on the basis when culture reaches 0.1 optical units at 600 nm with spectrophotometer (Schaad, 1992). 0.1ml of standardized suspension of the bacterial isolate (10^6cfu/ml) was spread inoculated on the nutrient glucose agar medium. 100µl of aqueous and 1 mg of solvent extracts were used separately in the wells made on sterile nutrient glucose agar medium (Raghvendra *et al.,* 2006;Gargade and Kadam, 2013). The plates were kept in refrigerator for 30 min. for diffusion and then incubated at 30ºC for 72 hs in an incubator.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of plant extracts showing significant antibacterial activity against *Xanthomonas axonopodis* pv. *punicae* were determined by broth dilution method. For broth dilution tests 0.1 ml of standardized suspension of bacterial isolate (10^6 cfu/ml) was added in each tube containing different concentrations of plant extracts ranging from 100 µg/ml to 1000 ug/ml. All the experiments were confirmed by repeating three times for confirmations.

Gas Chromatography Mass Spectroscopy analysis

The extracts showing significant antibacterial activity against *Xanthomonas axonopodis* pv. *punicae* were subjected to GCMS analysis. It was carried out at Indian Institute of Technology, Bombay. Agilent 7890 instrument for GC and Joel Accu TOF GCV instrument for MS were used. The inert gas helium (99.99%) was used as carrier gas with the flow rate of 1 ml/min. HP5 column with specification length 30 mm, internal diameter 0.32 mm, film of 0.25mm and temperature limit 80 °C to 280 °C was used. The total run time of GC was 30minutes. The oven temperature raised from 80 °C up to 280 °C with the rate of 8 °C permin rise in temperature. The sample size of 1µl was injected through the injector. The MSwas taken at 70eV. Interpretation and identification of individual components were done by using National Institute of Standards and Technology (NIST) Library. The retention time, molecular weight, molecular formula, name of the components detected in the plant extracts were ascertained. (Kiruthika and Sornaraj,2011). (Sagwan, 2012)

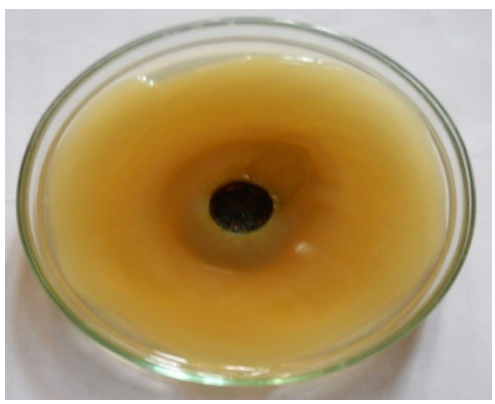
III. RESULTS AND DISCUSSION

Isolation and identification of the pathogen

The isolated organism was identified on the basis of morphological, biochemical characteristics and 16SrRNA analysis. s. The isolate used for Pathogenicity test showed the presence of oily black spots on the

leaves after incubation, indicates causative agent of bacterial blight of pomegranate. The bacterium was Gram negative, rod shaped, non-spore former, capsulated; cells appeared singly, in pairs, in chains. The results of morphological characteristics of the isolate were in agreement with the reports of Kanwar (1976); Shaad (1992); Manjula and Khan (2002); Yenjerappa (2009); Divya (2013); Basamma (2013); Katwal (2015).

The results of biochemical tests showed that the isolate had ability to produce acid and gas from sugars *viz.,* glucose, mannose, fructose, sucrose, dextrose. No acid and gas production was observed from arabinose, lactose, mannitol, and maltose, rhamnose. The isolate was negative for Indol, Voges-Proskauer, gelatin liquefaction, nitrate reductase, while positive for methyl red, citrate utilization, oxidase production, starch hydrolysis and hydrogen sulphide production. Similar results of biochemical characteristics of the pathogen have been reported by the earlier workers viz. Hingorani and Singh (1959); Chand and Kishun (1991); Schaad (1992); Yejerappa (2009); Pawar *et al.,*(2014); Digvijay *etal*.,(2014); Patel *et al.,*(2015).



P1: Methanol seed extract of *Acacia nilotica*

Antibacterial activity

The aqueous bark and methanol seed extracts of *Acacia nilotica* L. showed significant antibacterial activity against *Xanthomonas axonopodis* pv. *punicae*. **(Table 1) (P1)**. Aqueous bark extract, methanol seed extract of *Acacia nilotica* showed 35±1.00 mm, 30 ±2.88 mm diameter of zone of inhibition.

Table 1: *In vitro* antibacterial activity of *Acacia nilotica* (L.) against *Xanthomonas axonopodis* pv. *Punica*

Sr. No.	Plant used	Diameter of inhibition zone in mm (± SD)					
		Aqueous	Ethanol	Methanol	Ether	Benzene	Chloroform
1	*Acacia nilotica* bark	34 ±.1.00	3±1.00	8±0.57	--	--	--
2	*Acacia nilotica* seeds	6±1.52	14±2.51	30±2.88	6±1.52	8±2.00	10±1.15
3	Solvent	--	8±0.76	6±1.00	2±1.00	--	--
4	Streptomycin + COC	10±1.00	NA	NA	NA	NA	NA

(SD)-Standard Deviation, --: No antibacterial activity NA-Not applicable

Minimum Inhibitory Concentration of plant extracts

The MIC of selected plant extracts viz. aqueous bark and methanol seed extract of *Acacia nilotica* against *Xanthomonas axonopodis punicae* were 150 and 500µg/ml respectively.

GCMS chromatogram of aqueous bark extract of *Acacia nilotica*

Four compounds were found in aqueous stem bark extract of *Acacia nilotica* stem namely β-amyrin, Olean-12-ene, γ-sitosterol, β-sitosterol (Table 2).

Fig. 1: GCMS Chromatogram of aqueous stem bark extract of *Acacia nilotica.*

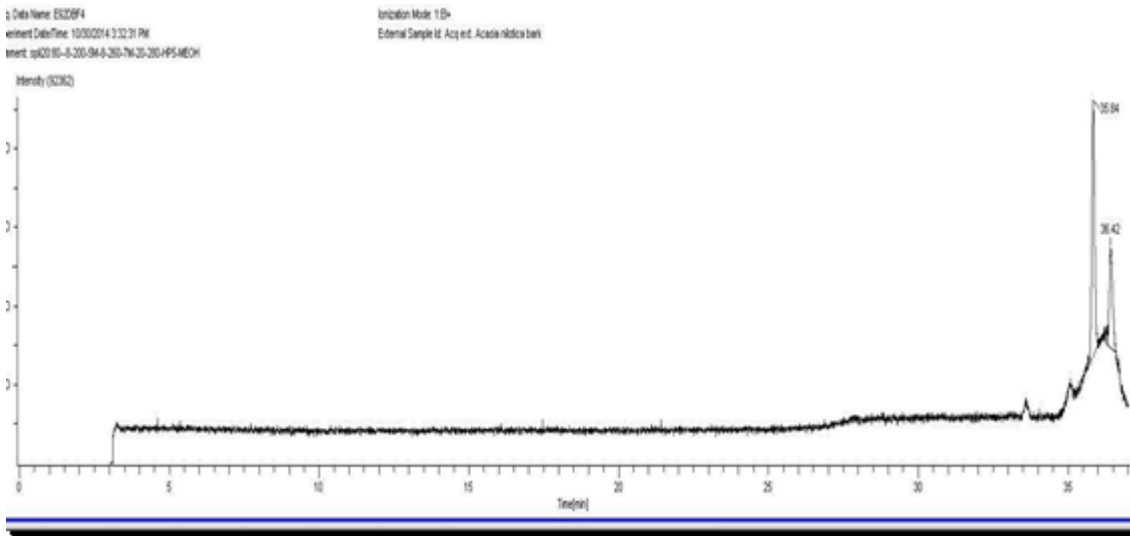


Table 2: Phytocomponents in the aqueous stem bark extract of *Acacia nilotica*

Sr. No.	Retention time(min.)	Molecular formula	Molecular weight (g/mole)	Compound
1	36.42	$C_{30}H_{50}O$	426.73	β-amyrin
2	36.42	$C_{30}H_{50}$	410.71	Olean-12- ene
3	35.84	$C_{29}H_{50}O$	414.00	γ-sitosterol
4	35.84	$C_{29}H_{50}O$	414.00	β-sitosterol

Phytochemical investigation of *Vernonia auriculifera* contains a sesquiterpene amine, lupenyl acetate, oleanolic acid, β- amyrin acetate, β-amyrin, friedelanone, friedelin acetate, α-amyrin and β-sitosterol. The compounds were characterized using Nuclear Magnetic Resonance (NMR) spectroscopy. The isolated triterpenoids (α-and β–amyrin) from *Vernonia auriculifera* exhibited moderate antibacterial activity against *Staphylococcus aureus, Bacillus subtilis*, *Enterococcus faecium* and *Staphylococcus saprophyticus.* Lupenyl acetate and oleanolic acid isolated from *Vernonia auriculifera* exhibited antibacterial activity against *Stenotrophomonas maltophilia*. Oleanolic acid decreased adhesion and formation of biofilm by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* These compounds showed potential for synergistic coupling with antimicrobial agents to improve therapeutic efficiency, in concern with increasing bacterial resistance. These observations were reported by Kiplimo *et al*., (2011).GCMS analysis of aqueous bark extract of *Acacia nilotica L*. revealed presence of similar compounds which are present in *Vernonia auriculifera*.

Sheema Bai *et al*., (2014) reported bioactive compounds from the chloroform leaf extract of *Acacia nilotica* L. by Gas chromatography and Mass spectroscopy (GCMS). The GCMS analysis revealed the presence of various compounds like 2, 4 dimethyl-butyl phenol, palmitic acid, linolenic acid, stearic acid, 2-methylresorcinol acetate,1,3,4 eugenol, megastigmatrienone, neophytadiene, myristic acid, lariciresinol, 3,4,7-trimethyl quercetin, δ-5-avenasterol and arachidonic acid.

GCMS chromatogram of methanol seed extract of *Acacia nilotica*

Eight compounds were found in methanol seed extract of *Acacia nilotica* namely 1, 2, 3-Benzenetriol, 1, 2, 4-Benzenetriol, 3, 5-Dimethyl-1-dimethylphenylphenylsilyloxybenzene, 1, 1, 3, 3-Tetramethyl- 1, 3-disilaphenalane, Myo-Inositol, 4-C-methyl, 3-O-Methyl-d-glucose, Benzoic acid, 3, 4, 5-trihydroxy-, methyl ester, 2, 5-furandicarboxylic acid, dimethyl ester **(Table 3).(Fig.2)**

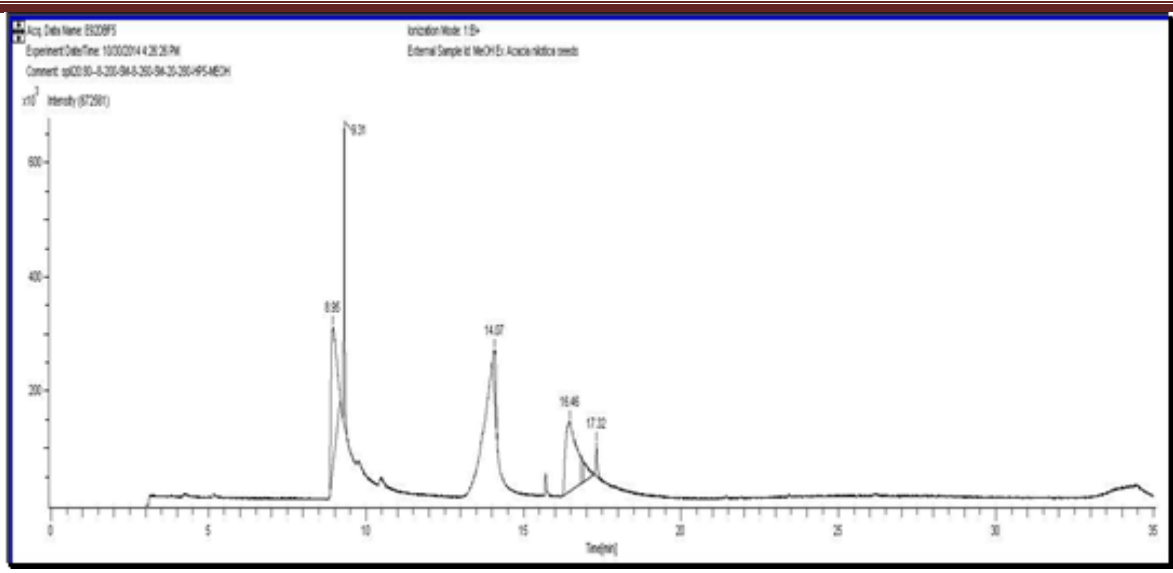


Fig 2: GCMS chromatogram of methanol seed extract of *Acacia nilotica*

Table 3: Phytocomponents in the methanol seed extract of *Acacia nilotica*

Sr. No.	Retention time (min.)	Molecular formula	Molecular weight (g/mole)	Name of the compound
1.	8.96	$C_6H_6O_3$	126.11	1, 2, 3-Benzenetriol
2.	8.96	$C_6H_6O_3$	126.11	1, 2, 4-Benzenetriol
3.	9.31	$C_{16}H_{20}OSi_2$	284.50	3, 5-Dimethyl-1-dimethyl phenyl phenyl silyloxybenzene
4.	9.31	$C_{15}H_{20}Si_2$	256.49	1,1,3,3-Tetramethyl-1,3 disilaphenalane
5.	14.07	$C_7H_{14}O_6$	194.18	Myo-Inositol,4-C-methyl
6.	14.07	$C_7H_{14}O_6$	194.18	3-O-Methyl-d-glucose
7.	16.45	$C_8H_8O_5$	184.14	Benzoic acid, 3, 4, 5-trihydroxy-, methyl ester
8.	16.45	$C_8H_8O_5$	184.14	2, 5-furandicarboxylic acid, dimethyl ester

Presently bacterial blight of pomegranate is controlled by using bactericides, Copper oxychlorides, micro nutrients, antibiotic Streptocycline. Present investigation is focused on use of medicinal plant extracts as an eco-friendly approach for management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae.* Use of plants as a source of medicine has been inherited and is important component of healthcare system. The secondary plant metabolites which are divided into different categories based on their mechanism function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial (Shariff,*et al.*,2006).

IV. CONCLUSION:

GCMS analysis of *Acacia nilotica* L. bark and seed extracts showed presence of bioactive compounds previously reported for its antibacterial activity. The use of *Acacia nilotica* L. plant extracts showed significant antibacterial activity against *Xanthomonas axonopodis* pv. *punicae* indicates that it can be used to control bacterial blight on Pomegranate. Results of this work will help to identify the compounds, which have antibacterial potential. Furthermore, the identification of plant metabolites is the first step to explain the benefits of traditionally used medicinal plants to control plant pathogen in future.

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COMPARATIVE ASSESSMENT OF LIGHT TRAPS FOR INSECT PESTS OF POMEGRANATE

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ABSTRACT: *Pomegranate is the one of the important cash crop of India, especially Maharashtra. The wonder crop is infected by several varieties of pest. Insects are found to be one of the major culprits causing severe damage to the fruits and as a whole to the crop. Chemical control methods pose side effects of accumulation of pesticides. Hence great alternative to the chemical control method is provided by light trap method. During present investigation Light traps using different light sources are used. Different light sources used were Mercury, CFL and LED. These light traps with different light sources were installed in the orchards of Pomegranate near Pandharpur city for observation.*

It is found during present investigation that light trap having mercury source of light was more efficient than other light sources. It has attracted most number of insects. Insects of different species were attracted towards light traps but the most attracted insect towards light trap among the insect pest of pomegranate were moths followed by aphids. Results are discussed with recent literature.

Keywords: *Pomegranate Insect Pest, Light traps, Light sources.*

I. Introduction

One of the very much important cash crops in India is Pomegranate. The intense reason behind prime importance of Pomegranate is the diverse and vital medicinal values of the wonder fruit.As the other cash crops, this crop also is infested by vast variety of pests including bacterial, viral, fungal and arthropod pests. Methods that are implied today mainly focuses on chemical control methods. But this method of pest control has more severe side effects including carcinogenic effects on the human body upon consumption of such fruits (Genuis and Kelln, 2015). Great alternative to the chemical control of insect pest is provided by the light trap method. Nomura *et al.* (1965) had explained first the efficiency of light trap method for control of insect pest. According to them use of lights in orchard had reduced damage of moths by about 50%.

Similar record of usage of light trap for insect pest control is carried out by various researchers like Rose *et al.* (2004). They had outlined the importance of light traps in controlling the Hawk Moths. Lee *et al.* (2008) had given a significant conclusion that Light Emitting Diode technology improves insect trapping methods.Ramamurthi *et al.* (2010) had studied the efficiency of different light sources in light traps in monitoring insect diversity.Abdul Rashid *et al.* (2012) had given an account of mechanisms of plant defense against insect herbivores. Thangalakshmi and Ramanujan (2015) had carried out studies on electronic trapping and monitoring of insect pests troubling agricultural pests.

Despite of the extensive studies on the light trap method of insect pest control there is lacuna in these studies particularly with the reference of Pomegranate Insect pest. Present investigation is an attempt to assess comparatively the efficiency of light traps in controlling pomegranate insect pest.

II. MATERIALS AND METHODS

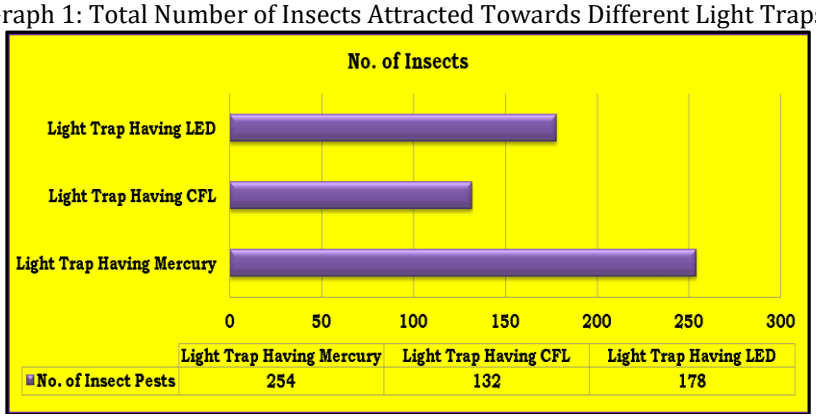
During present investigation collection of insects was carried out by the method prescribed by Hienton (1974). The present investigation was carried out in the monsoon months i.e. from June to September 2018 in the agricultural fields near Pandharpur City. The light traps were prepared as per the requirements. The light trap constituted collecting chamber, funnel, light source and lid from the top. For comparative analysis three different components were used as light sources. In one of the light trap mercury source having wavelengths 435.8nm was used. In the second light trap CFL (Compact Fluorescent Light) having wavelengths 400nm was used and in the third light trap LED (Light Emitting Diode) having wavelengths 360nm was used. These three light traps were installed in the same agricultural field for comparison. They were installed in the orchard of pomegranate near Pandharpur city at co-ordinates 170 48’ 16.72” N along latitude and 750 25’ 22.02” E along longitudes and 458 m above mean sea level.

III. RESULTS AND DISCUSSION

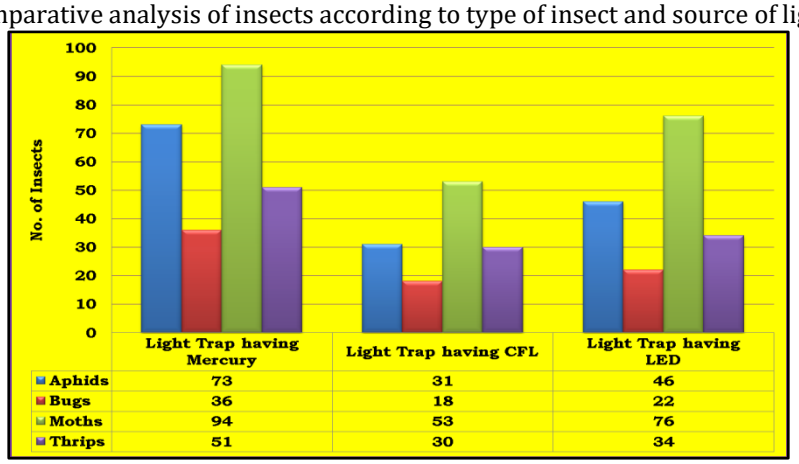
Results obtained during present investigation are presented in Graph 1 and Graph 2. During

Present investigation, three light traps were installed at three distinct sites in the orchards of Pomegranate. It has observed that insects like Moths, Thrips, Bugs, Aphids, Dragonflies, Drosophila, Hymenopteran flies, Mosquitoes etc. were attracted towards the light from the adjoining areas. Out of these, Aphids, Fruit Sucking Moths, Thrips and Bugs were Pomegranate insect pests. All the light traps were efficient enough to attract the insect pest of Pomegranate. Most insects were attracted by light trap equipped with mercury light. Comparatively light trap having CFL source of light were found to attract least amount of insects. Our findings are corroborating with the findings of Ramapurthy et al. (2010) and Thangalaxmi and Ramanujan (2015). Among the insects, prevalence of moths in all light traps was observed. Further moths were found to be attracted most to the light trap having LED source of light. Our results are in good agreement with the findings of Lee et al. (2008). Insect group that was least attracted towards light was aphids.

Graph 1: Total Number of Insects Attracted Towards Different Light Traps



Graph 2: Comparative analysis of insects according to type of insect and source of light trap.



IV. CONCLUSION

Observations of present investigation have revealed that Pomegranate Insect Pest gets attracted towards light and light traps can be useful in effective control of them. It is observed from present investigation that Severe Pomegranate insect pests like moths and thrips are also trapped by light sources. Light trap having mercury as a light source found to attract more insects as compared to other light sources used in present investigation. Fruit borer moths are attracted more by mercury light and LED light sources. Continuous monitoring and further experiments may reveal some additional aspects of efficiency of light trap in control of Pomegranate Insect Pest.

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MONITORING OF FRESHWATER BODIES OF MIRAJ TAHSIL WITH SPECIAL REFERENCE TO ENTOMOFAUNA

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ABSTRACT: *Diversity study is the major aspect in environmental assessment where invertebrates rank in their number and morphological aspects. Quantitative assessment of insects was carried out from various sites of the freshwater bodies of Miraj Tahsil in the period of January, 2011 to December, 2013. Diversified insects were observed in distinct groups as Coleoptera, Hemiptera, Orthoptera, Odonata, Dermaptera and Trichoptera. Among these, Odonata was found dominating with its eight species. Percent wise composition of insect diversity showed Odonata 39%, Hemiptera 28%, Coleoptera 11%, Orthoptera 11%, Trichoptera 6% and Dermaptera 5%. The obtained diversity data was discussed seasonal variation and water quality and was interpretated by applying stastical methods for calculation of diversity indices within the study region.*

Keywords: *Insect diversity, seasonal impact, freshwater bodies*

I. Introduction

Insects are the most diverse group of animals living on earth. Aquatic insects are a group of arthropods that live or spend part of their life cycle in water bodies (Arimoro and Ikomi, 2008). Insects are one of the most common groups of organism also used to assess the health status of aquatic ecosystems (Xu et al., 2014). Aquatic insects may be considered as model organisms in analyzing the structure and function of the inland waters because of their high abundance, high birth rate with short generation time with large biomass and rapid colonization of freshwater habitats (Sharma and Agrawal, 2012). Aquatic insect found important invertebrate organism both economically and ecologically.

Science of aquatic entomology includes Odonates, Plecopterans, Ephemeropterans. Trichopterans, Dipterans, Heteropterans, Hemipterans, Coleopterans etc as they spend a part of their whole life in aquatic system. Different aquatic insects have varying tolerances to a number of water quality parameters. Hence by proper study of aquatic insect diversity of the system, one can determine the status of a pond (Barman and Gupta, 2016).

Ecological studies highlighted the relationship between water quality and aquatic insects in Malaysia (Rasdi et al., 2012; Wahizatul et al., 2011 and Yap and Rahim, 2011). Considering the above literature and importance present work has been done.

II. MATERIALS AND METHODS

Materials carried during the collection were notebooks, data sheet, camera, insect sweeping net, wooden insect boxes, plastic containers, killing jars, brush, forcep etc.

Monthly samplings were made in the selected study sites from January, 2011 to December, 2013 over a period of 24 months. By using aquatic insects net, during early morning and afternoon hour's physico-chemical were recorded by applying (APHA, 1985). Collected samples were fixed in 70% alcohol in specimen bottles for further study. Collected specimens were brought to the laboratory for identification with the help of literatures of aquatic insects of India by Tonapi (1980), Pennak (1989), APHA (1998), Subramanian, 2005, Nair, (2011), Standard books and also from Zoological Survey of India (ZSI).

Species diversity indices such as Shanon -Weiner, Evenness were calculated to understand the diversity in biotic community of each study site. Shanon –Weiner diversity index (Shannon and Wiener, 1949) helps in species relative abun¬dance, evenness index is used for calculation of degree to which the abundances found equal among the species present in a sample.

III. RESULTS AND DISCUSSION

Several macrobenthic invertebrates have been used in assessing water. Nearly 3% of all the insects initiate their life cycle as aquatic larvae before emergence as winged ter¬restrial forms (Daly, 1996).

During the work assessment of insects showed total nineteen species belonging to six orders i.e. Odonata, Hemiptera, Coleoptera, Orthoptera, Dermaptera and Trichoptera. Among these order, Odonata was dominating with eight species belonging to three diverse families i.e Coenagrionidae, Libellulidae and

Aeshnidae. Order Hemiptera found second dominating group showing five species with three families i.e. Belostomatidae, Gerridae and Nepidae. Hemiptera was followed by Coleoptera and Orthoptera each one showing two species in which Coleoptera showed a single family i.e. Hydrophilidae and Othoptera showed two families i.e. Gryllidae and Gryllotalpidae. Order Dermaptera and Trichoptera each showed only single species belonging to family Forficulidae and Lepidostomatidae respectively. (Table. No. 1) Hemiptera, Odonata and Coleoptera are among the most regular insect orders in freshwater environments (Fontanarrosa et al., 2004; Martinoy et al., 2006 and Peralta et al., 2007) (Plate 1 to 5).

Percent composition of diversity in insect fauna showed order Odonata as dominating with 39% among the total count of species followed by Hemiptera 28%, Coleoptera and Orthoptera with 11%, Trichoptera 6% and Dermaptera with least dominancy i.e. 5% (Fig. No. 1 and 2). High rate of Odonata and Hemiptera indicated that freshwater habitats with rich oxygen content in it (Devi et al., 2013). Odonata are potentially considerable as indicators of environmental disturbance, especially by logging activities or pollution. They were found at site L2 and R2 which indicated that these sites were rich in oxygen and moderately polluted. Abundance and density of families belonging to the orders like Hemiptera, Coleoptera and Diptera was also noted (Bijita et al., 2014). The use of aquatic insects as bio indicators provided data to estimate the degree of environmental impact and its potential effects on other living organisms (Wahizatul, et al., 2011). Trichoptera and Dermaptera showed least dominancy. Trichoptera, found to be evolutionarily original from cold and temperate regions (Ross, 1967), tends to be more diverse in regions with similar conditions. Similar result was observed by Medona et al., (2015) at Sothuparai Reservoir, at periyakulam, Theni district, Tamilnadu.

A) Odonata:

About 5,000 species of odonates were found throughout the world. In India about 500 species and subspecies were reported. Their breeding habitats include both flowing and stagnant water bodies (Baruah and Saikia, 2015).

Odonates were found to be dominating and most diverse group with total 8 species during the study period. Among the five sampling sites Odanata was found to be more at lentic sites particularly at site L2 and L3. Gryllotalpa orientalis, Trithemus aurora, Crocothemis servilia, Pseudagrion decorum, Cratilla lineata, Orthetrum lyzonicum, Tholymis tillarga and Anax Sp. and Dragonfly larvae were s observed. Kulkarni et al., (2012) recorded 101 species of Odonate in Maharashtra. Gaurab et al., (2014) observed 48 species of Odonates from Manchabandha reserve forest, Baripada, Odisha; Muthukumarav et al., (2015) recorded 08 species of odonates in muthupet mangrove forest, Tamilnadu India; Dorji, (2014) recorded 24 species of odonates in Toebirongchhu sub-watershed in Punakha district, Western Bhutan; Sajan et al., (2014) observed 30 species of Odonata from in Palamau Tiger Reserve, Jharkhand, India. Bharamal, (2014) recorded 23 species belonging to 13 genera and 4 families from Sindhudurg district, Maharashtra, India; Rohmare et al., (2015) observed 42 species belonging to 27 genera, under seven families and two suborders from Central Gujarat, India.

The diversity of dragonflies species were found to be high during monsoon period followed by premansoon and postmansoon. The monsoon being the major factor in the density and distribution of plants leads to increase in abundance of herbivorous insects, the prey for Odonates Muthukumarav et al., (2015). Also the breeding season of Odonates in mansoon. The maximum odonate diversity was recorded in the rainy season followed by the summer and winter seasons (Nayak and Roy, 2016).

B) Hemiptera: (True Bugs, Cicadas, Hoppers, Aphids, and Allies)

Water bugs (Hemiptera), in general, are effective predators of varied aquatic organisms. Their role in nature may be both beneficial as well as harmful. They are beneficial in predating upon the larvae of noxious insect like mosquitoes, gnats, midges, etc., which are responsible for various kinds of human-diseases (Sharma and Agrawal, 2012).

Hemipterans were found to be second dominating group with total 5 species during the study period. Among the five sampling sites, Hemiptera was recorded at lentic as well as lotic site particularly at site L1 and R1. The species observed were Diplonychus rusticus, Diplonychus sp., Metrocoris brevis, Ranatra sordidula and Laccotrephes griseus. A list of 133 species of heteropteran bugs collected from the Maltese Islands was recorded by Attilio and David, (2015); 8 species were recorded from Turkey Mehmet et al., (2014); 53 species of assassin bugs belonging to 29 genera by Kailash et al., (2014) from Madhya Pradesh, India; 19 species of aquatic and semi aquatic heteroptera from some regions of Northern Iran by Ghahari, (2013); 32 species were identified from the Loktak Lake of Manipur, North East, India Devi et al., (2013). Kalita, (2008) recorded nine Hemipteran species from Deepar Beel, Ramsar sites in Assam, while Chetri et

al. (1997) reported seven species from the same wetland; Deepa and Rao (2007) recorded eight heteropteran Hemiptera from Pocharam Lake, Andhra Pradesh.

Hemipteran diversity was found to be maximum in mansoon season as compared to pre and postmansoon which may be due to decreased water level that has increased up with onset of rain in premonsoon and was reported maximum in monsoon. During fresh flow of water these bugs breed in large numbers and thus new generation gets populated (Dodson, 2008).

C) Coleoptera (Beetles)

Aquatic beetles including the true or diving water beetles, the whirligig beetles and the water scavengers beetles (Sharma, et al., 2013). Aquatic bugs and beetles were recorded in almost every freshwater biotope. They had many morphological adaptations to their aquatic environment (Barman and Gupta, 2016).

Coleopterans were found to be distinct group with 4 species during the study period. Among the five sampling sites, Coleopteran were found to be more at site L3 and R1. Hydrophilus sp. and Berosus sp. were the two genera collected from the sites belonging to family Hydrophilidae. Family Hydrophylidae are water scanvenger beetles and generally occur in shallower regions with abundant macrophytes particularly emergent ones and feed mainly on detritus, algae and decaying vegetative matter (Khan and Ghosh, 2001). Fifteen water beetles belonging to the family Dytiscidae, Hydrophilidae, Noteridae were recorded from the Loktak Lake, Manipur by Devi et al., (2014); three species were observed by Choudhary and Ahi (2015) at lakes of Madhya Pradesh; 19 species of beetles belonging to 10 genera were found by Dadmal and Khadakkar, (2014) at Akola, Maharashtra; 40 species collected in and around Pune by Sheth and Ghate, (2014); 24 species collected by Shayeghi et al., (2014) from Zayandeh Roud River, Iran; 36 beetles from Romania by Costae et al., (2013); Examination of 1,506 specimens of Coleoptera revealed 43 genera distributed in 13 families by Segura et al., (2007) in the state of Sao Paulo, Brazil. 5 species were observed by Vasantkumar and Roopa, (2014) in the pond ecosystem in Karwar, India.

D) Orthoptera (Grasshoppers, locusts, katydids and crickets)

The Orthoptera have inhabited the Earth for 300 million years and today include about 25,000 described species. The order Orthoptera is one of the largest having over 20,000 species worldwide with about 10% of the total world species 1,750 species recorded from India. Although orthopterans were mainly known to the general population by their most prominent species such as grasshoppers, locusts, katydids and crickets, which include an amazing diversity of forms and life-styles Bidau, (2014). At high population densities and under certain environmental conditions, some grasshopper species can change colour and behavior and form swarms (More and Nikam, 2016).

Orthopterans were found to be with two species during the study period. Among the five sampling sites Orthoptera was found at site L1. The species observed were Gryllus assimilis belonging to family Gryllidae and Gryllotalpa orientalis from family Gryllotalpidae. A number of orthopterans live underground but are not burrowers and comprise a number of cavernicolous or hypogean species (Vandel, 1965). All truly cavernicolous orthopterans belong to Ensifera and are included in two families: Raphidophoridae and Gryllidae. Many Researches worked on the diversity of orthopteran as Capello et al., (2013) had observed 17 species of floodplain lakes of the Paraná River; Distribution of 4 species of grasshoppers were noted by Adis et al., (2007) for Latin America and the Caribbean Islands; Mimicry and ultrastructural analogy between the semi-aquatic grasshopper had studies by WiIheIm et al., (1994); Feeding habits of 7 orthopteran species associated with macrophytes were studied by Capello et al., (2012) analyzed from the Middle Parana River, Argentina; 3 species were observed by Thamarai et al., (2015) from Sugarcane field at a Vadipatti, Tamil Nadu.

E) Dermaptera (Earwigs)

The common name, earwig, seems to have originated from myths concerning with these insects: that they occasionally enter the ears of sleeping humans and even penetrate the brain; or that the expanded wings of the earwigs resemble somewhat the shape of the human ear (Essig, 1942 and Imms, 1957). The Dermaptera are a small insect order containing about 2000 species (Sakai, 1996). Earwigs found generally similar in terms of the maternal care of eggs, incomplete metamorphosis, and the nocturnal habits (Langston and Powell, 1975). Earwigs are nocturnal, spending the day hidden under leaf debris, in cracks and crevices, and in other dark locations. Their nighttime activity is influenced by weather. Stable temperature encourages activity, and activity is favored by higher minimum temperatures but discouraged by higher maximum temperatures.

The earwigs are considered voracious predators because they have high ability to attack and feed on different preys, particularly eggs and immature stages of insects of the orders Lepidoptera, Hemiptera,

Coleoptera and Diptera. Thus, was found necessary to integrate the biological control of predator with control methods (Pasini et al., 2007).

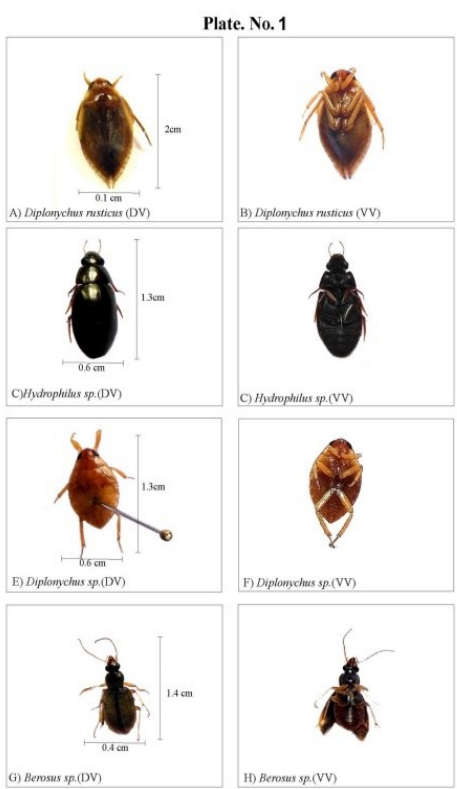
The single specimen observed from the family Forficulidae i.e. Forficula auricularia. Various Scientist had given their contribution to the study of earwig Capinera, (2013), studied European Earwig Forficula auricularia from Florida.

F) Trichoptera (Caddisflies)

Five Trichoptera families comprise 55% of the world's species and 19 families contain fewer than 30 species per family. Ten out of 620 genera contain 29% of the world's known species. Considerable underestimates of Trichoptera diversity for certain regions are recognised.

The only specimen observed from order Trichoptera belongs to the family Lepidostomatidae i.e. Lepidostoma sp. Some of the scientists have studieds Trichopteran order as, Sipahiler, (2000) studied new species and subspecies of Trichoptera from Turkey. Weaver, (2010) have studied the Caddisfly Family Lepidostomatidae (Trichoptera) in Vietnam; ITO, (2011) have studied six new species of the genus Lepidostoma Rambur (Trichoptera, Lepidostomatidae) from Japan; Parey and Saini (2013) had given two new species and two first records of the genus Lepidostoma (Trichoptera: Lepidostomatidae) from the Indian Himalayas; Parey and Saini, (2012) again described 4 new species of this genus from the Indian Himalayas. Aquatic insect, their diversity, distribution and assemblage are routinely assessed as an indicator of the health of running waters (Boulton and Lake, 2008). The distribution of aquatic insect functional feeding groups in running waters is supposed to reflect process-level of aquatic ecosystem. Specialized feeders, such as shredders and scrapers, are presumed to be more sensitive to perturbation, while generalists like gatherers and filterers, which were more tolerant to pollution as indicated by the availability of certain food (Barbour et al., 1996).

Insect diversity in selected sampling sites has been observed. Among the sites diversity was observed more at lentic sites as compared to lotic sites, especially at site L1 and L2 i.e. Brahmanath lake and Mhaishal lake. As order Odonata was found to be dominating, most of the species were found at L2 site. whereas order Orthoptera, Trichoptera and Dermaptra were observed at site L1 where rocks are present and hence the insects can hide in the crevices when out of water. Order Hemiptera and Coleoptera were observed at Site R1 i.e lotic site. Diversity was not observed at site L3 and comparatively less observed at R2 site as both the sites were found to be extremely polluted.



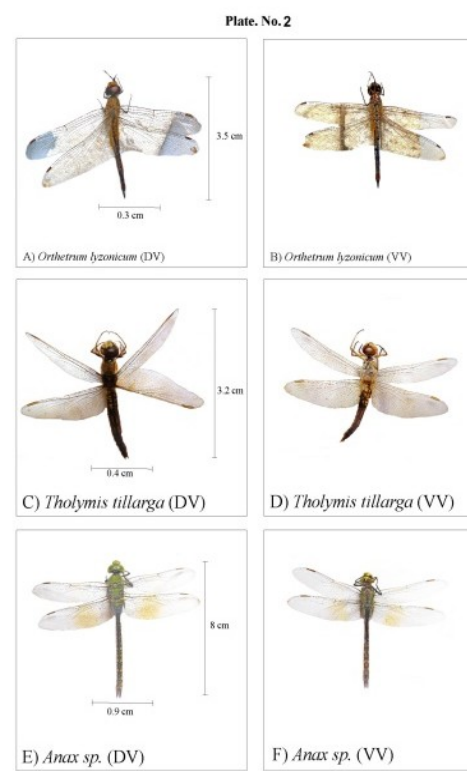
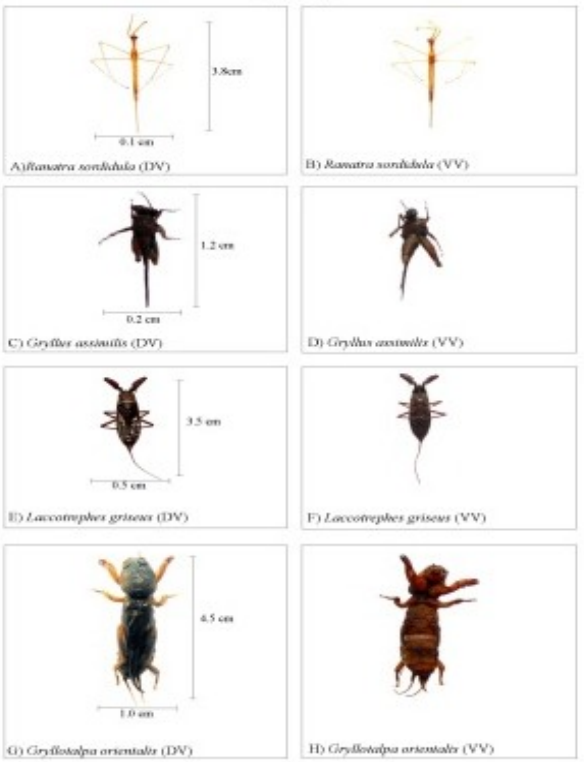


Plate. No. 3

Plate. No. 4



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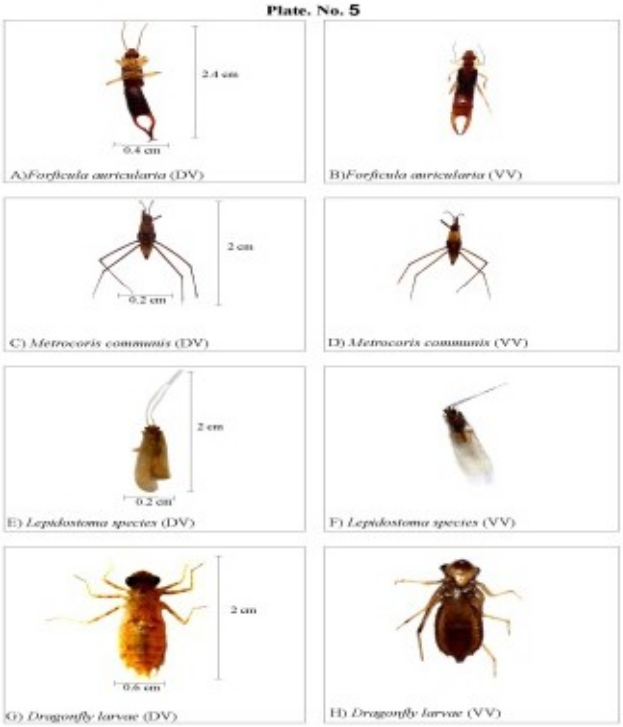

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INDUCED VARIATIONS IN THE ACTIVITY OF DIGESTIVE ENZYMES OF THE FRESH WATER FISH *CYPRINUS CARPIO* AFTER ACUTE EXPOSURE TO DIFFERENT PESTICIDES

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ABSTRACT: *India is agro based country, where large numbers of pesticides are widely used to control various agricultural pests. In the present study, organophosphate (OP) pesticide Triazophos, organochlorine pesticide deltamethrin and carbamate sevin is used to assess its impact on activity of digestive enzymes amylase, protease and lipase in intestine of common carp, Cyprinus carpio. The fish were exposed to 0.5 ppm (LC0) and 1.0ppm (LC50) concentrations of Triazophos, 0.0005 ppm (LC0) and 0.001 ppm (LC50) concentration of Deltamethrin and 1.0 ppm (LC0) and 6.0 ppm (LC50) concentration of sevin for 96 hrs. In present study, there was in general decrease in amylase, protease and lipase activity in intestine of test fish exposed to acute concentrations of these pesticides.*

Keywords: *Triazophos, Deltamethrin, Sevin, Amylase, Protease, Lipase, LC0, LC50, Cyprinus carpio.*

I. Introduction

Synthetic pesticides are diverse group of chemical compounds. The pesticides used today, belongs to four major groups of insectides. They are grouped as organochlorines, organophosphrous, carbamates and synthetic pyrethroids. They used in an extraordinarily wide range of setting. Due to this, dramatic increase in crop productivity was observed throughout the world. Pesticides are specially designed to be toxic to certain species, and this toxicity is basis of their utility (Hayes and Laws, 1991). However, major pesticides are also toxic to species beyond those targeted and have, consequently, caused severe damage to ecosystem. Pesticide chemicals are one of the important sources of aquatic pollution. Widespread effects of pesticide on environment have been studied by Edward (1971).

At present more than 1500 insecticides has been used throughout world. These are known as fungicide, herbicide, molluscicide, algaecide, acaricide, aphicide etc. (WHO, 2003). India having different patterns regarding use of pesticides. Nearly 76% pesticides are used in India as insecticides whereas; this utilization rate is 44% globally (Mathur, 1999).

An anthropogenic activity causes contamination, leading to pollution of water bodies. 'Water pollution is a major cause of global concern' (Daniel, 2006). Domestic, industrial, agricultural and shipping waste waters are the major sources of pollution. The problem of water pollution is more alarming for developing countries. More than 50% of water pollution of lakes, rivers and streams were carried out by chemicals used in the agricultural practices (Cook et al., 2012). Some of these pesticides are non-degradable or degrade slowly, and they are responsible for contamination of the ecosystem and enter the food chain. Fish occupies highest position in trophic level in food chain. Pesticide and other toxic content accumulation takes place easily in body of fishes as compared to other organisms. These pesticides due to their bioaccumulation and presence in food chain created serious problems in the aquatic ecosystem (Li et al., 2011).

Cyprinus carpio is one of the most cultured fish species in the world. In 2008, its world production was 29, 87,433 tons (FAO, 2011). It is omnivorous species feeding on planktons and benthos like worms, insects and mollusks, as well as detritus feeder in natural condition (Admek, 2004).The digestive system is well suited to a diet including more carbohydrate than carnivorous species. Common carp due to its presence in rivers and large scale use in aquaculture, it is taken as test animal for present study to assess the impact of pesticides on its digestive enzymes, though it is well accepted fact that toxicity, biochemical, histopathological, genotoxic studies and molecular changes in the fish tissue used as indicators for assessing degree of pollution caused by pesticides (Figen et al., 2013).

II. MATERIALS AND METHODS

The fresh water fishes were collected from Government fish breeding center, Kolhapur and brought to the laboratory for acclimation. The fishes used for experimentation were having average length of 6 to 9

cm and the weight of about 10-14 gms. After acclimation, the fishes were selected and divided into groups of ten fishes for exposer to toxicants. The pesticides used as toxicants are 1) Triazophos 40% EC 2) Sevin 50% EC and 3) Deltamenthrin 2.8 % EC.

Enzyme Preparation:

After acute (96h) exposure, the live fishes were sacrificed (5 from each group) for enzyme study. Intestine was quickly removed, blotted and weighed. The tissue was homogenized and tissue concentration was made to 10 mg/ml with 0.9% chilled NaCl. The homogenate was centrifuged at 3000 rpm for 10 minutes. Aliquots of supernatant were used as enzyme source.

Amylase:

The activity of amylase was determined by using 3,5-Dinitrosalicylic acid (DNSA) reagent (Bernfield, 1955). The free aldehyde group formed due to enzyme action on substrate reduces the DNSA reagent which was measured spectrophotometrically at 540 nm (Ishaaya and Swirski, 1970).

The assay consists of 1 ml of substrate (1% starch), 0.1 M. phosphate buffer of pH 7.4 / or appropriate buffer and 0.5 ml of supernatant as a enzyme source. Test tubes contain substrate plus enzyme was incubated at 40ºC for 10 min. The reactions were terminated by adding 2 ml DNSA and 2 ml of distilled water. The tubes were heated in boiling water bath for 5 min and then cooled. The developed colour was read at 540 nm. The standard curve for amylase was obtained by direct reaction with maltose using DNSA reagent under conditions similar to assay. The activity of enzyme was expressed as mg maltose /100 mg tissue / h and mg maltose / gr protein / hr. respectively.

Protease:

The protease activity was measured by Ishaya et.al, (1971) and Euguchi and Iwamoto (1982) method. The assay consist of 1.0 ml of 1% casein (Hammarstein) prepared in 0.1 M borate buffer of pH 7.6 and 0.5 mg supernatant as enzyme source. The test tubes were incubated at 37^0C for 30 minutes; to this mixture 3.0 ml of 5% TCA was added. The tubes were centrifuged for 10 min. 1 ml of supernatant and then 5 ml of Lowry's reagent was added with 0.5 ml of diluted follin phenol reagent. The absorbance of reaction mixture was measured at 660 nm against blank. The standard curve was obtained by using different tyrosine concentrations with similar conditions. The specific enzyme activity is expressed in terms of mg tyrosine / 100 mg tissue/ hr. and mg tyrosine / gm tissue/ hr. respectively.

Lipase:

The action of lipase was measured according to Hayashi and Tappel (1970). The assay consists of 0.25 ml substrate (triolene/olive oil), 0.25 ml of assay sample and 1.5 ml of Tris HCl buffer of pH 7.5. The assay mixture was incubated at 30 ^{0}C for 15 min. The reaction was terminated by adding 2 ml of ATC (mixture of acetic acid trielhanol amine and copper nitrate in ratio of 1:9:1) regent and 10 ml of chloroform, and reaction mixture was then vigorously shaken and allowed to settle for 1 hr. 1 ml chloroform layer (bottom layer) was pipetted out with syringe. Then 1 ml of freshly prepared lipase coloring reagent was added (25mg diphenyl carbazone +475 mg diphenyl carbazide in 100 ml methanol). Free fatty acids liberated in chloroform produce colour with reagent described earlier. Colour developed was measured spectrophotometrically at 550 nm. The standard curve was obtained by using palmitic acid under similar assay conditions. The total and specific enzyme activities were expressed as mg palmitic acid /100 mg tissue / hr and μg palmitic acid / gm protein / hr respectively.

III. RESULTS AND DISCUSSION

Amalyse:

Changes in the activity of amylase in intestine of fish *C. carpio* exposed to triazophos, sevin and deltamethrin for 96h (acute exposure) are presented in Table 1.0

There was decrease in amylase activity of intestine in *C. carpio* after acute exposure (96h) to various concentrations of pesticides triazophos, sevin and deltamethrin. The LC0 and LC50 values of these pesticides are 0.5 ppm and 1.0 ppm for triazophos, 1.0 ppm and 6.0 ppm for sevin, and 0.0005 ppm and 0.001 ppm for deltamethrin respectively.

After acute (96h) exposure to triazophos (0.5 ppm and 1.0 ppm), decrease in amylase activity was observed at both the concentrations. Significant decrease ($P<0.01$) in amylase activity was observed at LC50 concentration (2.89 mg maltose/100mg wet tissue/h), while less significant ($P<0.05$) change in amylase activity was recorded at LC0 concentration (3.25 mg maltose/100 mg tissue/h) as compared to control. Decrease in amylase activity was -31.30% and -22.72% at LC50 and LC0 concentrations respectively.

In sevin treated *C. Carpio* (LC0 and LC50 values), decrease in amylase activity was observed at both

the concentrations. Highly significant (P<0.001) change in amylase activity was recorded at 6.0 ppm concentration, while significant (P<0.01) change in amylase activity was observed at 1.0 ppm concentration. The decrease in amylase activity over control was -21.973% at 1.0 ppm and -31.781% at 6.0 ppm concentration.

After acute exposure of *C. Carpio* to deltamethrin, decrease in amylase activity was recorded at LC0 and LC50 concentrations. Decrease in amylase activity was highly significant (P<0.001) at 0.001 ppm concentration and significant decrease (P<0.01) at 0.0005 ppm concentration. As compared to other two pesticides, maximum decrease in amylase activity was observed at LC0 (-26.662%) and LC50 (-36.841%) groups after acute toxicity of deltamethrin.

Table –1.0. Effect of pesticides on amylase activity in intestine of fresh water fish *C.carpio* after acute exposure

Pesticide	Control	LC0	% change over control	LC50	% change over control
Triazophos	4.214 ± 0.368	3.256 ± 0.202 *	-22.722	2.895 ± 0.210 **	-31.304
Sevin	4.219 ± 0.226	3.292 ± 0.195 **	-21.973	2.878 ± 0.245 ***	-31.781
Deltamethrin	4.347 ± 0.338	3.188 ± 0.196 **	-26.662	2.746 ± 0.240 ***	-36.841

Values are mean ± S.D., *, ** and *** indicates significance level P<0.05, P<0.01 and P<0.001 respectively (n = 3)

Protease:

The effects of triazophos, sevin and deltamethrin on protease activity of the intestine of *C. Carpio* after acute exposure (96h) are presented in Table 2.

In fresh water fish *C. Carpio,* protease activity in intestine was found to be altered after acute (96h) exposure. Due to acute toxicity of triazophos after 96h, protease activity observed was 1.853 mg tyrosine/100mg tissue /h at 1.0 ppm (LC50) concentration while, at 0.5 ppm (LC0) concentration it was 2.067 mg tyrosine/100 mg tissue/h. Decrease in activity of protease was observed at both the concentrations as compared to control. At LC50 concentration, significant (P<0.05) change in enzyme activity was recorded while, at LC0 concentration, non-significant change in protease activity was observed. Percent decrease in protease activity as compared to control was -12.44% and -21.33% at LC0 and LC50 concentration respectively.

Due to sevin toxicity after acute exposure non-significant change in protease activity was observed at LC0 and LC50 concentration. The non-significant change in protease activity was -7.603% and -12.20% at (LC0) and (LC50) concentrations respectively.

As compared to control, after acute exposure of *C. carpio* to 0.0005 ppm (LC0) and 0.001 ppm (LC50) concentrations of deltamethrin, non-significant change in protease activity was observed at LC0 concentration while, significant (P<0.05) change was observed at LC50 concentration. Decrease in protease activity after acute exposure, in deltamethrin was relatively higher with -10.32% (LC0) -19.64% (LC50) as compared to other pesticides.

Table -2.0. Effect of pesticides on protease activity in intestine of fresh water fish *C.carpio* after acute exposure

Pesticide	Control	LC0	% change over control	LC50	% change over control
Triazophos	2.355 ± 0.127	2.067 ± 0.137 N.S.	-12.244	1.853 ± 0.174 *	-21.336
Sevin	2.296 ± 0.199	2.121 ± 0.132 N.S.	-7.603	2.015 ± 0.085 N.S.	-12.208
Deltamethrin	2.243 ± 0.196	2.011 ± 0.125 N.S.	-10.323	1.802 ± 0.070 *	-19.647

Values are mean ± S.D., * indicates significance level and NS – Non significant, (n = 3)

Lipase:

Alterations in the lipase activity in intestine of *C. carpio* after acute exposure (96h) to triazophos, sevin and deltamethrin are presented in Table 3. After acute exposure to various concentrations of

triazophos, sevin and deltamethrin, decrease in lipase activity was observed. For acute exposure predetermined LC0 and LC50 values of the three pesticides were used, as described earlier.

Due to acute toxicity of triazophos depletion in specific lipase activity was observed at both the concentrations. Highly significant (P<0.001) change in lipase activity was observed at 1.0 ppm (LC50) concentration, while significant (P<0.01) change in enzyme activity was observed at 0.5 ppm (LC0) concentration. The decrease in lipase activity was -23.24% at LC0 concentration and -32.08% at LC50 concentration as compared to control.

Due to acute toxicity of sevin highly significant (P<0.001) change in lipase activity was observed at LC50 concentration, while significant (P<0.01) change in lipase activity was observed at LC0 concentration. Decrease in lipase activity as compared to control was -21.068% at 1.0 ppm and -30.80% at 6.0 ppm concentration. As compared to other pesticides, minimum decrease in enzyme activity was observed in *C. carpio* after acute exposure when treated with sevin.

In fresh water fish *C.carpio* after acute exposure to deltamethrin resulted in highly significant (P<0.001) decrease in lipase activity at 0.001 ppm (LC50) concentration and significant (P<0.01) decrease in lipase activity at 0.0005 ppm (LC0) concentration as compared to control. Percent decrease in specific activity of lipase enzyme was -24.260% and -34.697% at 0.0005 ppm and 0.001 ppm concentrations respectively.

Table - 3. Effect of pesticides on lipase activity in intestine of fresh water fish *C.carpio* after acute exposure

Pesticide	Control	LC0	% change over control	LC50	% change over control
Triazophos	4.292 ± 0.215	3.294 ± 0.224 **	-23.247	2.914 ± 0.230 ***	-32.089
Sevin	4.209 ± 0.211	3.322 ± 0.226 **	-21.068	2.913 ± 0.189 ***	-30.800
Deltamethrin	4.243 ± 0.317	3.214 ± 0.210 **	-24.260	2.771 ± 0.141 ***	-34.697

Values are mean ± S.D., **, *** indicates significance level P<0.01, P<0.001 respectively (n = 3)

After acute exposure of *C. carpio* to various concentrations of pesticides described earlier, significant decrease (P<0.01) was noted at LC0 concentrations and highly significant (P<0.001) decrease was recorded at LC50 concentrations of all the pesticides tested. Maximum decrease in specific lipase activity was observed in deltamethrin, while minimum decrease in specific lipase activity was observed in sevin, while moderate toxicity was shown by triazophos upon enzyme activity.

In present study, there was in general decrease in amylase, protease and lipase activity in intestine of test fish exposed to acute concentrations of these pesticides. The decrease in activity of enzyme depends on concentration of pesticide. This decrease in activity was more at higher concentrations (LC50) as compared to control. Decrease in amylase activity was more in deltamethrin treated fishes at LC50 concentration as compared to other pesticides. Relatively less decrease in amylase activity was observed in sevin at both the concentrations. In triazophos treated fishes maximum decrease in protease activity was observed at LC50 concentration, as compared to deltamethrin and sevin at the same concentration. Significant decrease in lipase activity was observed in LC0 treated fishes, and highly significant at LC50 concentrations of all pesticides. While, significant decrease at both the concentrations of triazophos and sevin. Similar observations were made by Kamble (1999), Deshpande (2000), Li et al., (2014).

The mechanism of inhibition of activities of enzymes, amylase, protease and lipase is not clearly understood. It may be due to denaturation of enzyme protein resulting from pesticide toxicity or pesticides may bind with the active site of enzyme and inhibit its activity (Bhattacharya and Mukherjee, 1975). Under stress conditions, organism needs extra amount of energy, which might be probable reason behind the augmentation of most of the enzymatic activities (Gaikawad, 1998). Decrease in enzymatic activity may be related to hormonal level in the fish body. There might be decrease in hormonal level which might have suppressed the enzyme activity in fish.

IV. CONCLUSION

Triazophos, sevin and deltamethrin, once entered in to the body and alimentary canal of test fish, brought some drastic changes in cellular architecture and enzyme profile, Enzymes are known for their

specificity, being protein they acts as catalyst in the cell. The specificity and enormous effectiveness are two major important features of enzymes. Studies on enzyme activity thus help a great deal in giving deeper insight into vital phase of metabolic pathways.

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HYDROBIOLOGICAL STUDY OF CHIKOTRA DAM BHUDARGAD, DIST:- KOLHAPUR (M.S.)

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ABSTRACT: *The study of physicochemical parameters of water from Chikotra dam Bhudargad was done during June 2017 to May 2018.The analysis shows that water from Chikotra dam shows seasonal variations. In present investigation it is found that dam water is slightly alkaline, moderately heard and with low value of dissolved oxygen. Water temperature varied from 190C to 390C being highest in the month of May and lowest during the month of November. The pH was found to be varying from 7.0 to 8.1 which were slightly alkaline. The Transparency values ranged between 70 cm to 120 cm. The TDS fluctuated between 100 to 165 mg/lit. In the month of May it was and 100 mg / lit and in the month of June it was 165 mg/Lit. During May least DO was recorded 5.0 mg/lit and maximum DO was seen during rainy season June i.e.8.5 mg/l. Total hardness ranged between 80 mg/l and 120 mg/l. Other parameters are also given in detail. The diversity of phytoplankton and zooplanktons was also observed in the Chikotra dam.*

Keywords: *Chikotra dam, Zooplankton, Physicochemical parameters.*

I. Introduction

Chikotra dam is constructed near village Zulpewadi and Begawade, which are about 15 km east to Bhudargad city. Water from this dam is being utilized by peoples of villages on the bank of the river chikotra. Being less resources of water, this dam generally fills up to 50 to 60 percent every year. The water from this dam is used for drinking and agriculture purposes. From December water mainly used for drinking purpose because water level remains 20 to 25 % only. Since water is used for drinking purpose it is essential to check the physicochemical properties of the water in every season.

Physicochemical parameters strongly influence the aquatic organisms and many of them serves as ecological indicators of water quality (Mishra 1999).ecological studies generally involves analysis of physicochemical parameters and reflects on status of the environment in connection with both biotic and abiotic factors (Munawar 1974). This is helpful in utilizing the resources in right manner in order to avoid the pollution and conserve the prosperity of biodiversity. Since there is constant interaction and exchange of mass and energy in an ecosystem, the quality of water becomes an important and dynamic entity. That is exactly why the ecological studies have done on water from Chikotra dam during period June 2017 to May 2018.Similar studies were done in India by Dwivedi and pandey (2002), Hosmani et al.(1999),Kaur et.al.(2000) and in Maharashtra by Kolekar and Lohar (2012).

Table - 01. Salient features of chikotra dam bhudargad.

1	Name of the Dam	Chikotra (Kolhapur) dam
2	River	Chikotra
3	Nearest City	Bhudargad
4	District	Kolhapur
5	State	Maharashtra
6	Basin	Krishna
7	Purpose of Dam	Irrigation
8	Year of Completion	2001
9	Operating and maintenance agency	WRD,GOM
10	Seismic Zone	Seismic Zone -III
11	Type of Dam	Earthen
12	Length of Dam	983 meter
13	Maximum height above foundation	64.08 m
14	Total volume content(TCM)	5109
15	Spillway capacity	393 cumec
16	No. of Spillway Gates	3

II. MATERIALS AND METHODS

Total ten physicochemical parameters were considered monthly during the study period of one year from June 2017 to May 2018. Temperature, PH and transparency of water studied on the spot. Selected dam was visited 1st day of every month. At the sampling site, temperature is measured with thermometer. The pH was measured on the spot using pH paper and later confirmed in the laboratory using digital pH meter. Transparency was measured with the help of secchi disk . The water samples were brought to the laboratory for physicochemical analysis in separate plastic cans. Samples were collected for analysis in laboratory to find remaining parameters. Analysis of parameters was carried out according to the standard methods. The dissolved oxygen (DO) content of water was determined and primary productivity was measured. The seasonal variations in terms of primary productivity of the selected site of Cikotra dam in Kolhapur District were determined. The values are expressed as mg/Lit. for DO, TDS, and Cm for Transparency.

III. RESULTS AND DISCUSSION

In Chikotra dam it is clear that with increase in water temperature, DO and primary productivity decreases. Similarly it indicates that higher water temperature decreases primary productivity. Because of the shallowness of the lake, the temperature of water varies, as slightly lower or higher than those restricting maximum photosynthetic activity of phytoplankton. The records show variation in temperature, light intensity, DO and primary productivity during the day time in the month of June 2017. The Transparency values ranged between 70 cm to 120 cm. In rainy season transparency decreases, in the month of June it measures up to 70 cm and during summer it becomes 120 cm in the month of May. The TDS fluctuated between 100 to 165 mg/lit. In the month of May it was and 100 mg / lit and in the month of June it was 165mg/Lit.

The experiments were conducted between 10.00 am to 5.00 pm. Temperature and light intensity remained changing during the this period. After 2.00 pm the light intensity and temperature decrease, but DO level increased in the evening. However, the primary productivity remains high in the morning hours and low afterwards. It was perhaps due to low light intensity. The variation in temperature and light intensity shows variation in primary productivity. Roughly the change in primary productivity was parallels to the change in temperature. The maximum water temperature was noted (390C) in May and minimum (190C) in November. The present study indicates that temperature and Light intensity both vary during the experimental period. There are records of variations in dissolved oxygen and primary productivity (mg/l/h) for selected site.

There selected site from Chikotra dam in Kolhapur District (Maharashtra), The DO values ranged from 5.0 mg/l to 8.5 mg/l during all seasons. The highest value of 8.5 mg/l is recorded in monsoon and lowest 5.0 mg/l in summer. In general DO is lower during summer. The lower level of DO during summer may be due to higher temperature. The utilization of oxygen by micro-organisms found high levels of dissolved oxygen during monsoon. The selected site in dam show highest primary productivity during summer season. The pH recorded at that time goes to 8.1 which is slightly alkaline (Shown in Tables 2.)

The present study was done on the ecological features of the tank special reference to phytoplankton and zooplankton population. Presence of abundant phytoplankton and zooplankton indicates high level of productivity of the dam.(Nazneen.S.1980)The physicochemical parameters of the reservoir are well under the prescribed limits for inland surface water and can be used for drinking and for irrigation purpose.Graph 1: Total Number of Insects Attracted Towards Different Light Traps

Table - 02: Monthly variation in physicochemical parameters of chikotra dam in kolhapur district from June 2017 to May 2018.

Months	Air Temp.	Water Temp.	PH	DO	Dissolved solids	Trans parency	Alkali nity	Chlorides	Hard ness	Free carbon dioxide
June 17	27	21	7.1	**8.5**	**165**	**70**	**20**	**4.0**	**20**	**2.0**
July 17	27	20	7.2	10.5	150	80	30	5.5	25	2.5
Aug. 17	28	20	**7.0**	9.5	140	85	30	8.5	34	2.2
Sept. 17	29	22	7.9	9.6	120	87	45	6.0	38	3.0
Oct. 17	**26**	20	7.5	9.7	120	75	50	12	39	3.4
Nov. 17	29	**19**	7.6	10.1	120	90	60	18	33	2.9
Dec. 17	29	20	7.3	8.5	130	100	75	22	40	3.0

Jan. 18	32	25	7.4	7.8	115	99	79	33	60	3.7
Feb. 18	32	28	7.5	7.7	115		80	30	64	3.9
March 18	40	32	7.5	7.8	110	88	99	38	80	4.0
April 18	40	32	8.0	6.5	110	89	110	40	99	4.2
May 18	**42**	**39**	**8.1**	**5.0**	**100**	**120**	**120**	**46**	**114**	**4.6**

Table - 03. Physicochemical parameters

Sr. No.	Parameters	Range
1	Air Temperature	26 to42^0 c
2	water temperature	19 to 39^0 c
3	PH	7.0 to 8.1
4	DO	5.0 to 8.0 5mg / Lit.
5	Total dissolved solids	100 to. 165 mg / Lit
6	Transparency	70 to 120 cm
7	Total alkalinity	20 to 120 mg / Lit
8	Chlorides	4.0 to 46 mg/ Lit.
9	total hardness	20 to 114 mg / Lit.
10	Free carbon dioxide	2.0 t0 4.6 mg / Lit.

IV. ACKNOWLEDGEMENT

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STUDIES ON MORPHOMETRIC MEASUREMENTS OF FRESH WATER FISH, *ROHTEE COTIO* FROM RIVER GODAVARI, NANDED DISTRICT

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ABSTRACT: *In the fish biology the morphometric study has its own importance, as it helps to understand the relative growth of different body parts with the total length of the fish.With a view to find out the relationship between the dimensions of external parts of the fish and the total length of Rohtee cotio a series of measurements were taken.The regression analysis was made separately for each character to establish their relationship with the total length.*

Keywords: *Morphometric study, Rohtee cotio, Regression*

I. Introduction

The statistical relationship between total length of fish and various body parts is very useful to confirm their ratios commonly used in taxonomic and growth studies. The most reliable characteristics to identify fish species are provided from these studies. In some species the morphometric study is used for studying different races.

The method developed by Fisher (1930) and firstly applied by Mottely (1936, 1941) to some fishes in Canada is followed in this study and the terminology developed by Huxley and Teissier (1936) and Huxley, Needham and Lerner (1941), Snedecor (1946), Martin (1949), Hart (1952) and other demonstrated ontogenic and phylogenetic trends in fishes subjected to environmental and geographical variations using the constants.

In India morphometric studies of some economically important fishes have been carried out by Chacko and Krishnamurthy (1950), Pillay (1954), Prabhu (1955), Sarojini (1957), Radhakrishnan (1957), Ganguli et al. (1959), Dutt (1961), Seshappa (1970), Sugan and Sankaran (1972) and Somvanshi (1976).

With a view to find out the relationship between the dimensions of external parts of the fish and the total length of *Rohtee cotio* a series of measurements were taken.

II. MATERIALS AND METHODS

The fishes were collected regularly month wise from Budhwar fish market, for twelve months period from November 2017 to October 2018. The possibility of shrinkage was carefully considered and hence the fishes were measured with the help of a pair of engineering divider and a graduated scale in cm immediately after bringing them to the laboratory.

The following morphometric characters were examined to study changes in the body forms - Total Length, Standard Length, Head Length, Diameter of the Eye, Pre Dorsal Length, Pre Pectoral Length, Pre Pelvic length and Pre Anal length.

III. RESULTS AND DISCUSSION

In order to know the relationship between any two morphometric measurement, the statistical methods proposed by Snedecor (1961), was used in the present investigation to correlate the variables viz. 1) Standard Length 2) Head Length 3) Diameter of Eye 4) Pre Dorsal Length,5) Pre Pectoral Length, 6) Pre Pelvic length and 7) Pre Anal length with the Total Length, the linear regression was used.

$$Y = A + B (X)$$

Where Y = Variable, X = Total Length A = Constant B = Regression coefficient.

The values of constants A and B were calculated by the following formula,

$$B = \frac{\sum XY - NXY}{\sum X^2 - NX^2}$$

$$A = \frac{\sum Y - b\sum X}{N}$$

Where, N = Number of groups.

The regression analysis was made separately for each character to establish their relationship with the total length as follows –

1) Total Length (X) and Standard Length (Y)
Y = (- 0.0675) + 0.8082 (X)
2) Total Length (X) and Head Length (Y)
Y = (- 1.14) + 0.259 (X)
3) Total Length (X) and Diameter of Eye (Y)
Y = 0.195 + 0.047 (X)
4) Total Length (X) and Pre Dorsal Length (Y)
Y = 0.402 + 0.84 (X)
5) Total Length (X) and Pre Pectoral Length (Y)
Y = (- 1.795) + 0.306 (X)
6) Total Length (X) and Pre- Pelvic Length (Y)
Y = (- 1.555) + 0.426 (X)
7) Total Length (X) and Pre- Anal Length (Y)
Y = (- 3.061) + 0.645 (X)

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STUDIES ON BIOCHEHICAL CONSTTIUENTS OF HAEMOLYMPH AND HAEMAYTOLOGY IN *ANTHERA MYLITTA* DRURY

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ABSTRACT: *The present study was undertaken to recognize and study the hemocytes in Anthererea Mylitta Drury. Light and phase contrast microscopic observation have showed seven distinct types of hemocytes; Prohemocytes (PRs), Plasmocytes (PLs), granulocytes (GRs), Spherulocytes (Sps), adipophemocytes (ADs), oenocytoids (ODs) and coagulocytes (Cos). Total and differential counts of hemocytes were studied in 4th and 5th instar larva.*

Keywords: *Hemolymph and hemoatology, Anthererea Myllita Drury*

I. INTRODUCTION:

India is one of the rich biodiversity centers among the twelve mega biodiversity centers of the world.Floristically India is very rich, harboring three mega centers of endemism i.e. Western and Eastern Himalayas and Western Ghats. It is a treasure house of several diverse sericigenous flora and fauna. Hence India is a unique place for rearing of all four types of silk moth i.e Mulberry, tasar, eri and muga silk. Among non mulberry or Vanya silks belongs to the wild silk moths tasar. The tasar culture is practiced by about 1.5 lakh tribal in the States of Jharkhand, Chhattisgarh, Orissa and Madhya Pradesh. Tasar culture involves collection of nature grown coccons from forests or rearing of silkworm on its host plants in forests or rose by rearers for production of cocoons, which are utilized by reelers and weavers for production of yarn and fabrics. Tasar silkworm fed on various kinds of plant hence called as polyphagous.

Hematological studies are very essential in insect physiology. In insect open type of circulatory system is present. It circulates pale colored fluid called as haemolymph. It contains various types of cells called as haemocytes. The haemocyte performs various physiological functions in the body of insects. The haemolymph is a carrier of all nutrient substances distributes to each and every part of the body for cellular metabolism where the micromolecles are converted into complex macromolecules such as proteins and carbohydrates. The haemolymph is a circulating medium travels through haemocoel with bathing the all body organ. It contributes about 15 to 40% of insect volume. The volume of haemolymph depends on species as well as physiologically conditions of insects (Walshe, 1947 and 1950). The fluid phase, plasma composed of many organic and inorganic substances such as protein, lipid, free amino acids, carbohydrates and uric acid. Florkin and jeuniax (1974) reported ions in the haemolymph of insect such as copper, aluminum, zinc, manganese etc. They provide direct nutrients to various tissues and stored them also. Haemocytes provides phagocytosis, encapsulation of foreign bodies in the insect body cavity, coagulation to prevent loss of blood, nodule formation and transport of food materials and may be hormones and detoxification of metabolites and biological and may be hormones and detoxification of metabolites and biological active materials (Patton, 1983). Arnold and sohi (1974) studied five categories of haemocytes viz., prohemocyte, plasmatocyte, Granulocyte, Spherulocyte and Oenocytoid in the fresh blood of Malacosoma disstria H.

Circulating haemocyte play important role in defence mechanism against microorganisms in the hemocoel. Different types of hemocytes have been studied in insects by their morphological, cytological and functional characteristics. Monoclonal antibodies are used to precisely characterize hemocyte type and also to distinguish different subpopulations of these cells (Mullett et al., 19991; Lavin and Strand, 2002). Several workers have been studied the type and structre of haemocytes, ultrastructure of insect haemocytes and their function. Haemocytes have been studied mostly in Lepidoptera, Hymenoptera, Coleoptera, and Diptera (Gupta, 1985).

The plasmatocytes or prohemocytes are differentiated in haemopoetic organ reported by Gardiner and Strand, (2000). The granlocytes and plasmatocytes are only capable of adhering to foreign surface and non adhesive spherulocyte cells, oenocytoids and prohemocytes (Sass et al., 1994, Lackie; Ratcliffe and Strand) in Lepidoptera. Oenocytiod play importat role in melanization of hemolymph (Ashida; Iwama and jiang). Yamashita and Iwabchi (2001), however, suggest that B. mori prohemocytes are able to differentiate into plasmatocytes, granular cells, or spherule cells.

The normal haemocytes study gives information to physiologists, toxicologists and biochemists. It will provide basic knowledge to the entomological study. Insect haemocytes respond to internal changes during development such as starvation, wounding parasitism, diseases, and chemicals.

The physiology and biochemical activities of silkworm depend on the quality of food as well as availability of nutrient such as carbohydrates, proteins, lipids, amino acids glucose, vitamins and minerals. The digestive physiology and feeding habits is correlated with their food in all animals. Nutritional efficiency in larval stages significantly influences the resulting pupae and adult particularly in lepidopteron insects where in adult is a non-feeding stage (Srivastava et. al., 1982). Proteins,carbohydrates and lipids are fundamental biomolecule in living body. Haemolymph acts as storage reservoir for many materials essential for insects and its composition. The proteins are biomolecule plays a fundamental and physiology role in growth and development of silkworms and synthesis of silk proteins in silk gland during larval development. The proteins play an important role in the haemolymph of insect not only in specific transport functions, but also in their enzyme action, Hurliman and Chen (1974) and Chen (1966) asserted that the synthesis and utilization of haemolymph proteins are conditioned by genetic and hormonal control. The fat bodies are main source of haemolymph proteins and others may come from haemocytes. The proteins are the architectural blocks for body construction. Carbohydrates and lipids are essential components for energy demand during non feeding stage.

II. MATERIALS AND METHODS:

MATERIALS:

The 4th and 5th instar larvae of A. mylitta was used for study the biochemical changes and haematological study. The seeds were procured from Bhandara Grinage. The larvae of A. myllitta were reared in rearing house of Zoology department, Shivaji University, Kolhapur. The rearing was done as per the standard rearing methodology suggested by Krishnaswami et al (1978, 1979). Even though an A. mylitta larva is polyphagos, they fed on Terminalia catalpa in our rearing house.

The fresh and nutritive leaves were fed to the 4th and 5thinstar larvae twice a day. These larvae were used for further investigation in the laboratory. The larvae were anesthetized with chloroform and dissected in chilled ringer solution. The haemolymph was collected by cutting the prolog in pre- chilled and pre- coated of phenylthiourea. It was used for further study.

METHODS:

Preparation of sample:-

The haemolymph was collected from fourth and fifth instars larvae of A. mylitta. The larvae were mild an anesthetized with chloroform. The haemolymph of larvae were collectd by puncturing proleg in vials which of haemolymph was calculated and stored at 20°C for further estimation of proteins, carbohydrates, lipid, amino acid, cholesterol, glucose and ascorbic acid.

1) Estimation of total protein by Lowrys method:s

The total protein concentration n haemolymph of 4th and 5th instar larvae of A. mylitta were estimated by Lowry et al., (1951) method. To plot the standard graph of protein was used a as standard of BSA protein and made its solution. For calculation the, Bovine Serum Albmen (BSA) 5gm dissolved in 5ml of 2N NaOH and called as standard protein solution. In the blank and unknown tubes taken 0.5ml Distilled water and homogenate. In each the 5ml Lowry's 'C' solution was added and allowed undisturbed for 15min. Then 0.5ml folin – phenol reagent was added (diluted with distilled water in 1:3 ratio in each tube) and kept it for 30 mintues. The blue colour density of each tube was measured on spectrophotometer at 660nm. The standard graphs plotted with known values of BSA and determined the actual amounts of protein from each instar.

2) Estimation of carbohydrates by Neolting and Bernfeild's method (1948):

The pre-weighted haemolymph diluted with 2N NaOH and used as a sample. Three clean and dry test tubes were taken and 1ml sample was added in each tube then added 2.5ml DNSA reagent (10 gm of dinitrosalicylic acid dissolved in 200ml of 2N NaOH. Then added 500 ml distilled water. 300gm sodium potassium tartarate equalize to one liters volume with distilled water) and 2.5 ml distilled water. The reaction mixture was heated n boiling water bath for 5 minutes. The developed colour was read at 530nm on spectrophotometer by adjusting blank. The standard glucose values are used for the calculation of haemolymph carbohydrate.

3) Estimation of total lipid by Barnes and Blackstock method (1973):

For lipid estimation, pre- weighted haemolymph extracted in Folch's mixture (Folch's et. al., 1957).

The folch's mixture was prepared by taking chloroform and methanol in 2: 1 ratio. For standard graph known concentration of cholesterol were used for calculation. The unknown tubes contained 0.5 ml sample which was evaporated at 40C in water bath and then added 1ml of concentrated sulphuric acid. The tubes heated in boiling water bath for 10 minutes. After cooling 2ml of vanilline reagent were added in each tube. The pink colour developed was measured at 540m on spectrophotometer. The standard graph was plotted against unknown samples and calculates the actual amount of lipid.

4) Estimation of Amino acid:

The collected haemolymph was used for the estimation of Amino acid. The haemolymph was diluted and concentration was 1mg/ml used for further analysis. 0.2ml diluted haemolymph was taken in a three test tubes. And make up the volume 2ml by distilled water. Add 1ml ninhydrin reagent (Dissolve 0.5 gm of ninhydrin in 100 ml of ethyl alcohol forms a stock. Take 50 ml stock solution mix with 50 ml 4 M acetate buffer [pH 5.6] Stored in brown bottle. Protect from light 4.0 gm of sodium acetate + 21 ml Acetic acid in 100 ml of distilled water)

5) Estimation of cholesterol by Zak Method:

To calculate the amont of haemolymph cholesterol, 0.5 ml diluted haemolymph was taken in three dry and clean test tubes. The volume of each tube was adjusted 2ml by using alcohol and added 4ml cholesterol reagent(40ml iron reagent (2.5 gm of $FeCl_3 6H_2O$ in 100 ml conc. H_3PO_4) and 460ml conc.H_2SO_4. Waited for 30 minutes and O. D. was taken at 530nm against the blank. The standard graph (Standard Cholesterol. The cholesterol expressed in µg/ml.

6) Estimation of Ascorbic acid:

In 100ml conical flask, 5ml working standard solution (100µg/ml) was taken. In that added 10ml 4% oxalic acid and titrated against the Dye (42 mg of sodium bicarbonate and 54 mg of 2, 6 dichlorophenol indophenols dissolved in 200 ml of distilled water) and record as V ml. The amount of ascorbic acid was calculated by using standard values of ascorbic acid.

7) Estimation of Glucose:

The haemolymph glucose was estimated by method in 4th and 5th instar of A. mylitta. For the estimation following easy was followed. Diluted haemolymph 0.3 ml was taken into the three dry and clean test tubes of respective larvae. The volume was made 2ml with distilled water. The 2ml working glucose reagent (Glucose enzyme reagent quantitatively transfer the contents to a bottle and reconstituent with 50ml glucose dilutant mix slowly and dissolve completely) was added in each tube mixed well and incubated at 37°C for 10 minutes. The developed pink colour was read on spectrophotometer against blank at 510 nm. The amount of haemolymph glucose was calculated by using standard graph of glucose (1ml standard glucose + 10ml distilled water i.e 1mg / ml).

III. RESULT AND DISCUSSION:

Hematological count:

Total haemocyte count and differential haemocyte count in 4th and 5thinstar of A. Mylitta. The THC (Total haemocyte count) was done in 4th and 5th instar and expressed in per mm3 while DHC (differential haemocyte count) counted in percentage and diversity of haemocytes.

They are classified into seven types as follows,

1) Prohaemocytes (PRs)
2) Granulocytes (GRs)
3) Adipohaemocytes (Adi)
4) Plasmatocytes (PLs)
5) Spherulocytes (SPh)
6) Oenocytoids (Oen)
7) Coagulocytes (Coa)

In silkworm the haemolymph protein concentration fluctuates during its development and found that the protein concentration was increased seven fold during last instar of larval life. The high protein concentration indicates that the greater metabolic activity of the tissue. Protein concentration was recorded highly variable between the different batches of haemolymph studied by Lokesh et al in 2013. Higher protein concentration was recorded in the hemolymph of F1 hybrid female larvae.

The haemolymph is a carrier of all nutrient substance distributes to each and every part of the body for cellular metabolism where the micromolecules are converted into complex macromolecules such as proteins and carbohydrates. The concentration of carbohydrates in tissue is in relation to the energy requirements for the metabolism. Higher amount of reducing sugars in the hemolymph is due the release of

cellular carbohydrate in to the circulatory medium this subsequently channelize to different tisses/organs of the body. Trehaloses, glucose, fructose and mannitol, these compounds are highly essential during different development stages of an organism.

Lipids have an important role In cellular structure as well as energy storage transport and metabolic control. Lipids are the main source of energy for several physiological processes like embryogenesis, metamorphosis and reproduction (Gibert, 1967; pant, 1984). Gupta and Pathak, (1984), reported in the fifth instar larva contains the highest amount of lipids.

The haemolymph biochemical constituents like total protein, free amino acids, trehalose, glycerol and glycogen were studied in larvae of Daba bivoltine ecorace of tropical tasar silkworm Antheraea mylitta Drury in order to differentiate between non- diapause destined (NDD) and diapauses destined (DD) generation by Mishra et al, (2009) and reported that female of DD generation had significantly higher level of protein and amino acids than male larvae of DD generation. During the spinning period biochemical constituents, especially,amino acid and proteins in the haemolymph determines the undergoing diapause in pupal stage. The amino acid concentration was higher in indoor rearing of A.mylitta Andra local ecorace.

From the overall observation of biochemical moieties suggest that protein, carbohydrate, Lipid, Ascorbic acid, Amino acid, Cholesterol and Glucose are gradually increased in haemolymph of Vth instar larva as compare to IV th instar haemolymph. Total lipid content, Amino acid and Ascorbic acid where maximum in both instar as compare to other contents.

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ASSESSMENT OF BODY MASS INDEX (BMI) AND BLOOD PRESSURE (BP) OF TEACHING FACULTY FROM VITA DIST. SANGLI (M.S.)

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ABSTRACT: *Around the world, India is rapidly developing country with accepting modernization with changing lifestyle. On other side, facing the problems of increased body mass, obesity, hypertension, cardiac problems, physical and emotional stress, etc. among population. Physiological condition and metabolic functions are directly relates with alterations in body mass index, hyperlipedemia, blood pressure and cardiovascular diseases. Perfect health status of can be controlled by regular monitoring of blood pressure, body mass index with cardiovascular parameters, which provides information about fitness of the person. With this aim, the present investigation was carried out by initial assessment of physiological indices as weight, Body Mass Index, Blood Pressure, physical fitness etc. The data obtained was statically interpreted for the physical fitness of the teaching faculty and correlated with the physical and emotional stress if any.*

Keywords: *Physiology, Body Mass Index, Blood Pressure, physical fitness*

I. Introduction

Developing country like India, industrialization and urbanization has uplifted standard of living in every sector Gundogdu, (2008) documented several pathological trends of overweight and obesity in relation to hypertension especially among urban population. In support to this, Wang et. al., (2009), reported increased prevalence of body weight and obesity over the past decade in India, where as in some urban and high socioeconomic groups it reached relatively at pick levels. Physiologically it has been observed that, generally problem of blood pressure arises as people get overweight and older. Age is known risk factor for high blood pressure (Mungreiphy et. al., 2011). Raj et. al., (2010), recorded that, high BP in adults can be originated from childhood and feeding behavior. As per clinical definition of blood pressure, it is the resistance of blood flow against the walls of the arteries (Martin et. al., 2015). With scientific evidences, blood pressure was classified as high or normal according to the classification of SBP (high ≥140 mmHg) and normal <140 mmHg, (Saxon et. al., 2010).

The relationship between BMI and blood pressure has long been the subject of epidemiological research. Body mass index (BMI) is a measure of the human body weight in relation to the height, calculated by dividing the weight of a person in Kg by the square of the height in meters (Sizer et. al., 2012). Srikanth et. al., 2011), documented that, obesity concern with health consequences and pathological burden, to reach epidemic proportions in developing countries like India. Many studies have found the relationship between blood pressure and age (both SBP and DBP) to be significant among both males and females. Diastolic pressure, however, is the pressure required to allow constant flow in the blood vessels and filling of the ventricles before the next systole (Saxon et. al., 2010).

Hypertension represents one of the most prevalent chronic conditions in the US, with normotensive middle-aged adults estimated to have a 90% residual lifetime risk of developing hypertension (Vasan et. al., 2002). Hypertension is associated with the incidence of stroke, coronary heart disease, congestive heart failure and renal insufficiency (Lauer et. al., 1989). Srikanth et. al., (2011) observed that, obesity or excess relative weight is found to be associated with increased risk of disease morbidity and mortality (Tyagi et. al., 2007). JNC, (2003), reported several studies carried out in different parts of India on factors affecting cardiovascular functions and associated problems, even reported death. Obesity is associated with sympathetic activation and is the leading risk factor for development of hypertension, (Rahmouni et. al., 2005). Small prospective studies have also reported links between abdominal accumulations of body fat with the risk of hypertension, (Cassano et. al., 1990).

By taking account of factors playing a significant role, such as consuming too much calories of fatty food, coupled with lack of physical exercise and inability to burn out the excess calories, overall results in becoming overweight. So we have decided to assess the relation between body mass index and its relation to the blood pressure in the academic sector, teaching faculties (mental working field) from Vita, District

Sangli, India. Results obtained were interpreted for the risk of development of hypertension and associated physiological problems in the subjects.

II. MATERIALS AND METHODS

For the present investigation, teachers working in the academic sector (mental working condition) were selected. About 80 faculties from Balwant College, vita, district Sangli, India were subjected for physiological assessment. All the selected staffs were asked for Body mass index assessment by applying standard method prescribed by Kaur, (2016). Observations of weight and height of faculty were carried out by properly calibrated weighting balance and height meter respectively. The BMI was calculated by using following formula-

$$\textbf{BMI} = \frac{Body\ Weight\ in\ kg}{Height\ (Meter)^2}$$

For the differentiation, as per the data, of Body Mass Index was subjected various categories as underweight, normal, overweight and obese individuals by using the BMI chart provided by WHO.

Range	Below 18.5	18.5-25	25-30	Above 30
Type	Underweight	Normal	Overweight	Obese

In addition to this subjects were asked for manually measurement of another associated parameter as blood pressure using Sphygmomanometer in sitting position after 10 minutes of rest. All the readings were accurately measured as per the standard norms and with prier information to all staff members and for compilation all procedure was repeated thrice.

III. RESULTS AND DISCUSSION

Most of the studies showed the relationship of BMI and blood pressure against cardiovascular diseases. Assessment of Body Mass Index (BMI) recognized as one of the most recommended indices for obesity assessment in adults. Martin, (2015), pointed out the parameters related to causes of obesity. Sobngwi et. al., (2004) documented that; life style in the urban environment has relation with obesity, diabetes, and hypertension. Lack of physical activity and working in continuous sitting posture leads to cause overweight and obesity as noticed by, (Winnick et. al., 2016). Edwards et. al., (2000) explained some of the, hypertension prevalence and care in an urban and rural area of Tanzania. Similarly, prevalence, awareness, treatment and control of hypertension among the elderly in Bangladesh have been critically notified by, (World Health Organ, 2001). Hu, (2008), carried out work in relation to obesity and reported cardiovascular abnormalities in the less physical workers. Saxon et. al., (2010), studied role of nervous system to control the physical change and aging mechanism and interpreted the prevalence of associated cardiac problems. Similarly body fat distribution, blood pressure and hypertension found to be prospective factors of normative aging study as stated by, (Cassano et. al., 1990). Mufunda et. al., (2006), also focused on causes of hypertension and its relationship with obesity, they reported results of national blood pressure survey in Eritrea and concluded that static work is responsible for obesity and related cardiac problems.

Ng et. al., (2006) documented methodologies for assessing epidemiological transition and assessment of combining risk factor and demographic surveillance in the people suffering from the cardiovascular diseases. In the present investigation we found that, physiological parameters like height, weight, sex, physical activity with body mass index and blood pressure has some relation for the development of obesity, hypertension and associated cardiovascular problems. After assessment of teaching faculty we noticed correlation between systolic blood pressure and BMI and diastolic blood pressure and BMI. Among the faculty, minimum BMI recorded was 16.4 while maximum BMI recorded was 39.8. The maximum systolic blood pressure recorded was 160 mmHg, while minimum systolic blood pressure recorded was 110 mmHg. With respect to this, maximum diastolic blood pressure was 99 mmHg and minimum diastolic blood pressure was 69 mmHg.

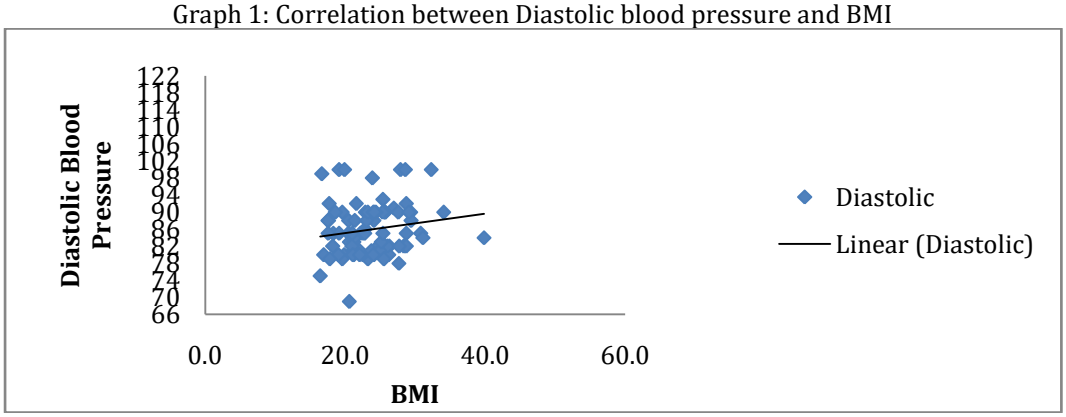
Statically observed sex differencing body mass index readings showed that minimum number (2) of male faculties were underweight. Average (24) male faculties were having normal body mass index. Nirmala, (2001), reported age variation in blood pressure and its effect of sex and urbanization in a genetically homogeneous caste population of Andhra Pradesh. We found that, about 11 males faculty members were overweight and facing the problems of obesity. On the other hand 16 female faculty members were underweight, 17 female faculty members were normal BMI and 6 female faculties were showed overweight and obesity. Cohen et. al., (2016) carried out relationship between Body Mass Index and Intraocular Pressure in Men and Women.

Table No. 1 Sex differencing body mass index of Faculty members.

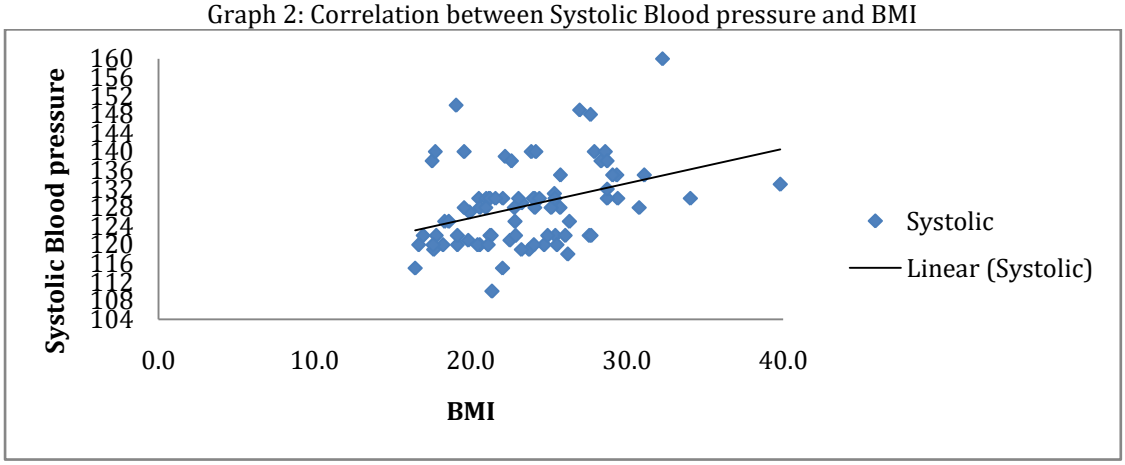
BMI Categories	No. of Faculties		
	Male	Female	Total
Underweight	2	16	18
Normal	24	17	41
Overweight	11	6	17
Obese	2	2	4

Statistical analysis of BMI and blood pressure revealed that there is correlation in between these two Parameters. Kapoor, (2000) documented correlation between blood pressure, waist to hip ratio and body mass index among affluent Punjabi girls of Delhi. Both systolic and diastolic blood pressure are positively correlated with the BMI i.e. with increase in BMI, blood pressure may also increase. The pattern of correlation in between these two is depicted in the form of scatter diagram in Graph No. 1 and 2 respectively.

Graph 1: Correlation between Diastolic blood pressure and BMI



Graph 2: Correlation between Systolic Blood pressure and BMI



Yusuf et. al., (2004), recorded effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries and interpreted that working condition even light physical work and psychology has direct relation for cardiovascular diseases. Mungreiphy et. al., (2011) noticed association between BMI, Blood Pressure and age while study among Tangkhul Naga Tribal Males of North east India. In support to this, it was found that there is relationship between blood pressure and anthropometry in a cohort of Brazilian men as reported by (Cassani et. al., 2009). Mungreiphy and Kapoor (2009) reported emerging epidemic features of obesity: health consequences, assessment and its implication and its relevance's in Obesity.

O'Donnell et. al., (2010) investigated risk factors for ischaemic and intracerebral aemorrhagic stroke with concern to overweight and obesity problems. Similarly He et. al., (2000) documented long-term effects of weight loss and dietary sodium reduction on incidence of hypertension and its physiological problems in the office workers. It was found that, Obesity and its physiological pattern has adverse health implications among Khatri population, (Tandon 2006). Tyagi and Kapoor (2004), concluded that, ageing in structural and functional dimensions among institutionalized and non institutionalized senior citizens have impact to the body mass index, obesity and development of healthy risk like cardiovascular problems. Whelton et. al., (2002), carried out some primary prevention of hypertension associated with the different parameters of clinical and public health advisory concern.

IV. CONCLUSION:

Present investigation enlights that body mass index is closely associated with both systolic and diastolic blood pressure. We noticed that, some degree of correlation among BMI, age, systolic and diastolic blood pressures. The staff of the study area found to going for facing the cardiovascular problems due to increasing percentage of overweight and working stress. So, present study concludes that maintains BMI in normal range is a key factor for physical fitness. Routine health monitoring and anthropometric surveillance are needed to maintain good health and to avoid possible adverse consequence of health. Regular physical and mental exercise becomes mandatory for to maintain the physical fitness.

V. ACKNOWLEDGMENT:

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FECUNDITY OF GOBIUS STRIATUS FROM WESTERN MAHARASHTRA (SOLAPUR DISTRICT)

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ABSTRACT: *Fecundity is reproductive capacity of a fish determined by the number of eggs stored in each spawning season and its knowledge is extremely important in successful management and exploitation of its fishery.*
All together 10 specimen of Gobius striatus were examined with a view to determine the average number of ova produced by each species and also to find out the relationship between fecundity and variables such as total length, body weight, gonad length and gonad weight of the fish.
The estimated fecundity of Gobius striatus is ranged from 3135 to 5005.

Keywords: *Fecundity, Western Maharashtra, Gobius striatus, Population dynamics.*

I. Introduction

Fecundity is reproductive capacity of a fish determined by the number of eggs stored in each spawning season and its knowledge is extremely important in successful management and exploitation of its fishery.

Studies on fecundity are receiving much attention as they play a key role in fish stock management. This is the most important aspect of fishery biology.

Fecundity has been determined for many fishes, which provides information of population dynamics, racial characteristics, and production and stock recruitment problems.

The analysis of fecundity data in relation to size and weight of the fish has often been used to provide a reliable index of density dependent factors affecting the population size. Inhibition and reproductive process due to influence of physicochemical factors affects fecundity. A dense population of the fish brings in intra and inter specific competitions for food and reproduction.

Fecundity indicates the number of ova produced by the fish to form the crop of season. The number of eggs produced may differ in different species with differences in size and age of fish. The egg production capacity also varies in the different as well as in same species depending upon the length and weight of the fish and length and weight of gonad and also the locality from where the fish is caught.

II. MATERIALS AND METHODS

The material which formed the basis of the present study was collected from Ujani reservoir, Solapur District in Western Maharashtra during the year 2015 in months from July to September when fishes were predominantly ripe.

All together 10 specimen of Gobius striatus were examined with a view to determine the average number of ova produced by each species and also to find out the relationship between fecundity and variables such as total length, body weight, gonad length and gonad weight of the fish.The specimen ranging from 21 cm to 31 cm in total length were selected. Before dissecting the females the total length and weight of the female fish was noted. The ovaries in stage IV were preserved in 5% Formalin.

The ovaries after being hardened for few days removed from Formalin and surface moisture was blotted using blotting paper. The entire ovary was then weighed accurately to the nearest mg.

A small portion from the middle region of the ovary was then teased on a slide and few drops of Formalin were put on them so as to prevent the ova from getting dried and mature ova were counted under microscope. Care was taken to ensure that the ova were spread evenly in a single layer. From the number of ova obtained from the small portion of an ovary of known weight, the number of ova in the entire ovary was calculated on the basis of its total weight.

The fecundity estimates of all the specimens examined were made by egg counts and also from various variables viz. total length of the fish, weight of the fish, length of ovary and weight of ovary. The females ranged between 21 cm to 31 cm in length and 82.47 gm to 280.00 gm in weight, where as length of ovary varied between 4.20 cm to 6.20 cm and ovary weight between 2.90 gm to 4.65 gm. In Gobius

striatus the total number of ova varied from 3788 to 6556, (Table 8).

III. RESULTS AND DISCUSSION

The relationship between the fecundity of fish and length of fish, weight of fish, length of ovary and weight of ovary was observed. The relation between fecundity and length is non linear and suggests the following form of equation,

$$F = ALB$$

Where, F = Fecundity, L = Length, and A and B are two constants to be calculated from data.

LENGTH OF FISH AND FECUNDITY RELATIONSHIP: (Table 1):-

It has been shown by Heidnet (1975) and Kesteven (1942) that the number of ova holds some exponential relationship with the length of the fish in the same way as does the weight. According to Kesteven (1942), the gonad maintenance size relationship with the reminder of the body of the organism and since the size of the ova is constant. In general, the number of eggs, being in effect number of units of weight, will show an exponential relation with the length in the same way as does the weight of the entire animal. The relationship between fecundity and length of *Gobius striatus* is found to be linear.

It is revealved that from Table No.1 and Figure No.1, the estimated fecundity of *Gobius striatus* is ranged from 3135 to 5005. The relationship between fecundity and total length has been determined by least square method and is expressed as follows –

$$Y = 2.03 + 1.19 (X)$$

Where, Y = Log F (Fecundity) Number of Ova.

X = Log L (Length) Total length of fish in cm.

Franz (1910 a and b), Kesselewitch (1923) and Clark (1934) stated that the frequency increased with the square of the fish length. Farron (1938), showed that in the Irish Herring the increase in fecundity was 4.5 to the power of length. Hickling (1940), has found cube relationship between fecundity and total length of the fish. Lehman (1953), Peterson (1961), Parulekar and Bal (1961) and Desilva (1973) have reported the increase in fecundity rate more than the fourth power of the length.

Begenal (1957) ,stated that the relationship of fecundity with length of the fish is normally linear in nature. Antony Raja (1979) and Desilva (1973), have reported curvilinear relationship between total length and fecundity of fishes. Dahlgran (1979), found fecundity of *Poecilia reticulata* is decreasing with increasing population density.

It is clear from the equation that the fecundity of this species increases at the rate of 1.19 times the total length. It is further noticed that the fecundity – total length relationship is parabolic and the graph when plotted shows a linear path represented by the points for estimated fecundity values, the observed fecundity values are seen as encircled dots closely situated along the graph.

The result of the present investigation show that in *Gobius striatus* the fecundity increases at a rate less than square of its length.

The standard error of estimate was calculated by the formula,

$$Sd = \sqrt{\sum d^2 / n}$$

Where, SD = Standard error of estimate. $\sum d^2$ = the sum of the squares of the differences between observed and calculated values.

n = Total number of observations.

The standard error of estimate for total length is 3215.72.

Weight of Fish and Fecundity Relationship: (Table 2):-

The relationship between body weight and fecundity was found to be linear. A straight line relationship between the fecundity and body weight was observed by Begenal (1957), Sarojini (1957), Pillay (1958), Tondon (1961), Qasim and Qayyum (1963), Savant and Bal (1969), Parulekar and Bal (1971) and Bhowate (2002). The formula expressing the relationship between fecundity and weight of the fish was found to be,

$$Y = 2.283 + 0.68 (X)$$

Where, Y = Log F (Fecundity) and

X = Log W (Weight of fish)

Standard error of estimate is 2929.2178.

Length of Ovary and Fecundity Relationship: (Table 3):-
The relationship between fecundity and average length of the ovary was found to be linear. The equation is as follows –

$$Y = 1.990 + 2.38 (X)$$

Where, Y = Log F (Fecundity) and X = Log L (Length of ovary).
The standard error of estimate was found to be 2107.0256.

Weight of Ovary and Fecundity Relationship: (Table 4):-

Hickling (1940) stated that the egg production is the dominant function of ovary. Hence a close correlation is expected between the weight of ovary and number of ova produced.

Prabhu (1963) while working on some marine fishes from Bombay (Mumbai), observed a linear relationship between ovary weight and fecundity. Qasim and Qayyum (1963), while working on Ophiocephalus punctatus made similar observations. Savant and Bal (1969) and Parulekar and Bal (1971), have shown the rectilinear relationship between ovary weight and fecundity. Bhowate (2002), while working on *Clupeisoma bastari* has shown a linear relationship between ovary weight and fecundity. The relationship between ovary weight and fecundity was found to be as follows

$$Y = 2.586 + 2.00 (X)$$

Where, Y = Fecundity and X = Weight of ovary.
It is seen that fecundity and weight of ovary show a straight line relationship.
The standard error of estimate is 2542.7875.

Table No. 1 : Observed Fecundity of *Gobius striatus*

Sr. No.	Length of fish in cm	Weight of fish in gm	Length of ovary in cm	Weight of ovary in gm	Weight of part of ovary in gm	Number of ova in the part of ovary	Fecundity or observed number of ova
1	21.0	88.12	4.20	2.96	1.00	1280	3788
2	22.1	97.38	4.42	3.12	1.00	1300	4056
3	22.8	103.00	4.50	3.32	1.00	1322	4388
4	23.0	82.47	4.60	3.35	1.00	1326	4442
5	23.7	85.00	5.18	3.49	1.00	1330	4642
6	24.0	99.27	4.80	3.54	1.00	1344	4758
7	24.4	120.30	4.88	3.44	1.00	1338	4602
8	25.5	100.00	4.68	3.71	1.00	1420	5268
9	26.2	140.00	5.24	3.69	1.00	1408	5196
10	31.0	280.00	6.20	4.65	1.00	1410	6556

Table 2 : **Relationship between Length of fish and observed and calculated number of ova and standard error of estimate in Gobius striatus.**

Sr. No.	Length of fish in cm	Fecundity or observed ova	Log L	Log F	**XY**	**X^2**	Estimated or Calculated Log F	Estimated or calculated fecundity	Difference between observed and calculated number of ova
	L	**F**	**X**	**Y**			**Y**	**F**	**d**
1	21.0	3788	1.3222	3.5784	4.73	1.75	3.1350	3135	653
2	22.1	4056	1.3444	3.6080	4.85	1.81	3.3322	3332	724
3	22.8	4388	1.3579	3.6422	4.95	1.84	3.4602	3460	928
4	23.0	4442	1.3617	3.6475	4.97	1.85	3.4972	3497	945
5	23.7	4642	1.3747	3.6667	5.04	1.89	3.6255	3625	1017
6	24.0	4758	1.3802	3.6774	5.08	1.90	3.6805	3680	1078

7	24.4	4602	1.3874	3.6629	5.08	1.92	3.7512	3751	851
8	25.5	5268	1.4048	3.7216	5.23	1.97	3.9548	3954	1314
9	26.2	5196	1.4183	3.7156	5.27	2.01	4.0888	4088	1108
10	31.0	6556	1.4914	3.8166	5.69	2.22	5.0055	5005	1551
Total			13.84	36.74	50.89	19.16	37.53		10169

Table 3 : **Relationship between weight of fish and observed and calculated number of ova and standard error of estimate in *Gobius striatus*.**

Sr. No.	Weight of fish in gm	Fecundity or observed ova	Log W	Log F	XY	X^2	Estimated or Calculated Log F	Estimated or calculated fecundity	Difference between observed and calculated number of ova
	W	**F**	**X**	**Y**			**Y**	**F**	**d**
1	88.12	3788	1.9450	3.5784	6.96	3.78	1.7492	2749	1039
2	97.38	4056	1.9885	3.6080	7.17	3.95	1.9722	3750	306
3	103.00	4388	2.0128	3.6422	7.33	4.05	2.1096	3110	1278
4	82.47	4442	1.9163	3.6475	6.99	3.67	1.6169	3616	826
5	85.00	4642	1.9294	3.6667	7.07	3.72	1.6767	3676	966
6	99.27	4758	1.9968	3.6774	7.34	3.99	2.0190	4019	739
7	120.30	4602	2.0802	3.6629	7.62	4.33	2.5391	3539	1063
8	100.00	5268	2.0000	3.7216	7.44	4.00	2.0372	4037	1231
9	140.00	5196	2.1461	3.7156	7.97	4.61	3.0417	4047	1149
10	280.00	6556	2.4471	3.8166	9.34	5.99	6.8901	5890	666
Total			20.46	36.74	75.23	42.09			9263

Table 4 : Relationship between Length of Ovary and observed and calculated number of ova and standard error of estimate in *Gobius striatus*.

Sr. No.	Length of ovary in cm	Fecundity or observed ova	Log L	Log F	XY	X^2	Estimated or Calculated Log F	Estimated or calculated fecundity	Difference between observed and calculated number of ova
	L	**F**	**X**	**Y**			**Y**	**F**	**d**
1	4.20	3788	0.6232	3.5784	2.23	0.39	6.46	3646	142
2	4.42	4056	0.6454	3.6080	2.33	0.42	6.58	3658	398
3	4.50	4388	0.6532	3.6422	2.38	0.43	6.70	3670	718
4	4.60	4442	0.6627	3.6475	2.42	0.44	7.37	3737	705
5	5.18	4642	0.7143	3.6667	2.62	0.51	6.95	3695	947
6	4.80	4758	0.6812	3.6774	2.51	0.46	6.99	3699	1059
7	4.88	4602	0.6884	3.6629	2.52	0.47	6.86	3686	916
8	4.68	5268	0.6702	3.7216	2.49	0.45	7.49	4749	519
9	5.24	5196	0.7193	3.7156	2.67	0.52	8.73	4873	323
10	6.20	6556	0.7924	3.8166	3.02	0.63	6.20	5620	936
Total			6.85	36.74	25.19	4.72			6663

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STUDIES ON PHYSICO-CHEMICAL PARAMETERS OF FISH PONDS FROM HADPSAR, PUNE-28, (MS), INDIA

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ABSTRACT: *The aquatic organisms and are food for fishes. Polluted or contaminated water affects the productivity and quality of the ponds. Regular monitoring of physicochemical and biological parameters is essential to determine status of water bodies Physicochemical parameter of the well water used for breeding and spawning of major carps are showing less DO 2mg/lit and high hardness 95 mg/lit. whereas Khadkwasla dam water used for stocking ponds and nursery ponds are showing average temperature of 280c+/- 1.30c, more dissolved oxygen 7 mg/lit and hardness of 95 mg/lit. The physicochemical parameters of this pond water were analysed and recorded before breeding after release of hatchling and after manuring. The hardness of nursery and stocking ponds varies from 65 to 75 ponds the nitrates was varying from 1.499 to 1.625 mg/lit. The phytoplankton's were measured up to 5,500 / mm3 and zooplanktons up to 9,900/mm3 in stocking ponds. It was 22,000/mm3 and 55,000/mm3 respectively in nursing ponds after manuring. The results indicate that the pond water is suitable for fish culture.*

Keywords: *DO (dissolved oxygen), well water, carps, stocking ponds, nursery ponds.*

I. Introduction

In fish culture, productivity and ecology of ponds are very important aspects. Artificial or natural water body, which is covering approximately 1m2 and 2 hectares (~5 acres or 20,000m2) in area, can be considered as a pond. It holds water at least for four months of the year or more2. Water is the primary requisite for fish culture in ponds. Water offers most favourable conditions for the existence of not only the fishes but other aquatic organisms as well4. These aquatic organisms are food for fishes. Polluted or contaminated water affects the productivity and quality of the ponds. Contamination of water bodies takes place due to human and animal interference. This contamination of water bodies may change in their tropic status and may make them unsuitable for aquaculture. Various physicochemical or biological factors could act as stressors17. These factors may adversely affect fish growth and reproduction.13 Regular monitoring of physicochemical and biological parameters is essential to determine status of water bodies like ponds, lakes, etc., with reference to fish culture. To enhance the productivity in fish farm, water quality and soil condition of several of several fishponds in relation to fish production in India were studied.1 It is a fact that pollution of water deteriorates water quality and causes adverse effect on fish diversity and growth. Water quality in general means the components of water which must be present for optimum growth of aquatic organism .The determinant of good growth in water body includes dissolved oxygen, hardness, turbidity, alkalinity, nutrients, temp. etc. The parameters like biological oxygen demand (BOD), and chemical oxygen demand (COD), indicate pollution level of the water body, the fish population strongly influences by the environment variability8. In most water bodies, various chemical parameters occur in low concentration.

The concentration level increase due to human activities, and lack of environmental regulations. Government Fish Breeding Center, Hadapsar selected for present studies. Since 1958 rearing of Major carps using Chinese hatchery is carried out in this centre. The fish farm is located east Pune city over 14 acres between 18°57′ 57.30′′N latitude and 73 º 57′ 23.35′′ E longitudes in western India. The fish farm has Chinese hatchery center. It has incubation chambers; Chinese hatchery, two rearing ponds, 1 nursery ponds and 6 stocking ponds. The well water is taken in Chinese hatchery for breeding and spawning of carps, where as in rearing and stocking ponds, the water from Khadkwasla dam is taken. The Khadakwasla dam is located on the south of Pune city between 18º 26′ 21.12′′ N and 73 º 46′20.93′′ E. The water of Khadkwasla dam flows to the fish farm by open canal covering approximately a distance of 35 km. The dam water quality is threatened by the activities of local inhabitants alongside of canals through city by ways of waste discharge, bathing, washing, agricultural practices, etc. The study was aimed to establish that the physicochemical parameters of spawning pond (well water), rearing and stocking pond (Khadakwasla dam water) are suitable for fish culture.

II. MATERIALS AND METHODS

The water samples collected from the well during breeding and spawning seasons. Whereas water sample from two nursery ponds 13 and 14 and stocking ponds D and F were collected fortnightly, during study period. Water temperature recorded with the help of centrifuge thermometer in ºC while pH, chlorine and DO estimated by using rapid method kit of AQUAMERK. The nitrate and total hardness of water estimated. The total plankton was estimated by using Haemo-cytometer; density of planktons is estimated by Lackey's method.

Observations: Table 1: Observations before starting the breeding:

Pond / Parameters	Well	Pond 'A'	Pond 'B'	Pond 'D'	Pond 'F'	Pond '13'	Pond '14'
Dissolved Oxygen	2	4	4	7	5	7	7
pH	7.5	7.8	7.8	7.8	7.6	7.5	7.5
Temp(^{0}C)	27	29	28	28	29	28	28
Chlorine(mg/lit)	0.1	0.1	0.1	0.1	0.2	0.2	0.2
Free CO_2	0.4	0.3	0.3	0.3	0.3	0.3	0.4
Nitrates($NaNo_3$)(mg/lit)	1.625	1.675	1.632	1.6	1.732	2.004	1.735
Hardness($CaCO_3$)	85	90	75	65	65	68	66

Table 2: Observations of Hatchlings in Happa, Nursery ponds. (Fishes in pond 'D')

Pond / Parameters	Pond 'D'	Pond 'F'	Pond '13'	Pond '14'
Dissolved Oxygen	7	5	7	7
pH	7.9	7.6	7.5	7.6
Temp (^{0} C)	27	26	26	27
Chlorine (mg/lit)	0.2	0.1	0.2	0.2
Free CO_2	0.2	0.3	0.3	0.3
Nitrates($NaNo_3$) (mg/lit)	1.45	1.57	1.98	1.45
Hardness ($CaCO_3$)	75	67	68	65

Table 3: Observations after manuring the ponds: ((Fishes in pond 'D' and ' F')

Pond / Parameters	Pond 'D'	Pond 'F'	Pond '13'	Pond '14'
Dissolved Oxygen	7	5	7	4
pH	7.9	7.5	7.6	7.5
Temp (^{0} C)	27	26	26	27
Chlorine(mg/lit)	0.2	0.2	0.2	0.1
Free CO_2	0.5	0.4	0.3	0.3
Nitrates($NaNo_3$)(mg/lit)	1.632	1.6	2.004	1.735
Hardness($CaCO_3$)	75	67	68	65

Table 4: Observations after 45 days after hatching: (Fishes in pond 'D', 'F', 13, 14)

Pond / Parameters	Pond 'D'	Pond 'F'	Pond '13'	Pond '14'
Dissolved Oxygen	dry	5.	3.2	1.3
pH	dry	7.6	7.6	7.2
Temp (^{0} C)	dry	27	27	27
Chlorine(mg/lit)	dry	0.2	0.2	0.2
Free CO_2	dry	0.4	0.3	0.3
Nitrates($NaNo_3$)(mg/lit)	dry	1.62	1.99	1.1
Hardness($CaCO_3$)	dry	66	67	68

Table 5: Water samples in 1.5 litre from Pond 'F', 13, 14 (**Phytoplankton and Zooplankton**)

Sr. No.	Pond	Phytoplankton	Zooplankton
1	F	3750	6750
2	13	15375	37,000
3	14	2,250	17250

Table 6: Density of Phytoplankton and Zooplankton by Lackeys Method.

Sr. No.	Pond	Phytoplankton	Zooplankton
1	F	5500	9950
2	13	22000	55,000
3	14	3300	25300

III. RESULTS AND DISCUSSION

Dissolved oxygen: The main source is air and photosynthetic activity of aquatic plants. It is also dependent upon the physical, chemical and biological activities of water body. The main source of DO is atmosphere and photosynthetic activity of aquatic plants. It is also dependent upon the physical, chemical and biological activities of water body. In the present study it was found that the value of DO in well water is 2mg/lit. whereas in nursery pond 13 and 14, before manuring, it was 7mg/lit and after manuring it varied between 4mg/lit to 5 mg/lit. The DO in aquatic ecosystem brings various biochemical changes especially metabolic activities. Seasonal variation in DO i.e. minimum in winter was referred by [6]. In nursery pond 13 and 14 the DO is declining, as in table 4.0, after manuring. This may be able to increase in utilization of DO by zooplankton and fishes. It was found that zooplankton were 55000 as compared to phytoplankton 22000.

Temperature: Temperature is one of the most important among the external factors which has a profound influence, and direct and or indirect effect on biota of an ecosystem. In the well water temperature is comparatively lesser; moreover there are no aquatic plants. The temperature range varied from 26 ºC to 29 ºC during the study period. The temperature variation was very less to influence the fish production. It was observed that the pH and temperature have shown to influence the density and diversity of the aquatic communities, [5]. It was noticed that the temperature increases in summer and reduces in order seasons [11].

pH:pH is defined as the intensity of the acidic or basic range for pond pH is 6.5 – 9.5 [9], where as the acceptable range is 5.5 – 10.0[15]. pH values ranged from 7.5 in well water to 7.9 in nursery and stocking ponds. The pH values are slightly higher in stocking ponds (Table 1), presumably due to more production of metabolic waste, i.e., CO_2. Similar observation was reported earlier also[16]. We observed that the pH range belonging in tune with other studies referred above. It also helps in getting better production from the ponds.

Chlorides: The chloride values of the ponds were ranging from 0.1 mg/lit to 0.2 mg/lit. No remarkable variation was observed in chlorine during study period. It may be due to the water taken from the same water source, i.e., Khadakwasla dam.

Free CO_2: The value of free CO_2 was higher in the well water and was relatively lower in other in other ponds. It reduced after manuring, but indicated that it may increase after some period in the stocking dam. The free CO_2 should not be more than 25 mg/lit for better fish productivity[12].

Nitrates: The nitrate values in the nursery pond and stocking pond was observed ranging from 1.20 mg/lit to 2.004 mg/lit. This falls in the desirable limits, i.e., 0-2 mg/lit.[7]. Comparatively higher values of nitrates may be due to frequent manuring to the ponds for the plankton growth, which serves as food for fishes. It was recorded that lower level of nitrate is due to utilization of nitrate during photosynthesis[19]. The nitrate levels were found to supportive for fish culture [13].

Hardness: The total hardness value in the pond is the sum of calcium and magnesium hardness concentrations. The maximum hardness in well water was found 95 mg/lit and the hardness of nursery and stocking pond varies from 65 to 75 mg/lit. These values falls within the range of recommended values of 25-100 mg/lit [18]. The values of hardness in nursery and stocking ponds are comparatively lesser then the well water due to unpolluted nature of water. In industrial waste water the hardness was reported 2000mg/lit to 85.9 mg/lit[5]. In polluted water higher value of hardness were also observed[3]. Hard water enhances the productivity of planktons[14].

The physic-chemical parameters are important determinant of the quality of water. The results have been indicated in tabular formats as above observation tables.

IV. CONCLUSION

This is a quality assurance process to ensure that there are no toxic substances in the ponds leading to possible bio-accumulation and magnification. The regular analysis of pond water helps to maintain the higher fish productivity. The evaluation of water quality of Hadapsar fish farm indicates its suitability for breeding and rearing of fishes. Total number of ponds, capacity of breeding and spawning can be increased as the conditions are conductive/ favourable. It is observed that the farm favours successful fish culture of major carps.

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ANALYSIS OF PHYSICO-CHEMICAL PARAMETERS AT SELECTED SITES OF UJANI RESERVOIR, SOLAPUR DISTRICT, M.S. (INDIA)

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ABSTRACT: *Ujani Dam also known as Bhima Dam or Bhima irrigation project is the major freshwater aquatic ecosystem constructed on the Bhima river located near Ujani village of Madha tehsil in Solapur district, Maharashtra state. The Bhima river basin has about 14 tributaries having total drainage area of 48,631 Km2, it spreads over the Maharashtra (75%) and Karnataka (25%) states. The Ujani Dam and its large reservoir provide multi-purpose benefits including irrigation, drinking, hydro-electric power generation, Industrial water supply and fisheries.*

Present study was undertaken from Nov.2014 to Sept.2016 with respect to bimonthly seasonal variations in physico-chemical parameters. The selected sites are Ujani Dam, Bhima River (Down-stream), Shirala, Kandhar and Chikhalthan. Most of the selected sampling sites are very close to human population and agricultural land; hence their physico-chemical properties are greatly influenced by anthropogenic activities.

Keywords: *Ujani Reservoir, Bhima River, Physico-chemical parameters, Aquatic ecosystem, Anthropogenic activities.*

I. Introduction

Water is universal solvent as it dissolves more substances than any other liquid without undergoing any chemical change. Thus water; the unique component of nature has played an important role in the life. Water is required for all living components of the earth for their daily activities and any minute change in it may leads to harmful effects on the dependents. Ujani water reservoir is composite type of dam. The coordinates are 18º4´26´´N, 75º07´12´´E. The height of the dam is 56.4 m (185ft). The width (crest) is 6.7m (22ft). The dam volume is 33,20,000m^3. This reservoir is dispersed from Tembhurni to Khadki – Diksal. Ujani is the third largest reservoir in Maharashtra after Koyna (2800 GL, on Koyna - Krishna) and Jayakwadi (2171 GL, on Godavari). It was Constructed in June 1980 on Bhima river having gross Storage: 115 TMC, live Storage: 55 TMC and area at FRL: 32500 ha (about).

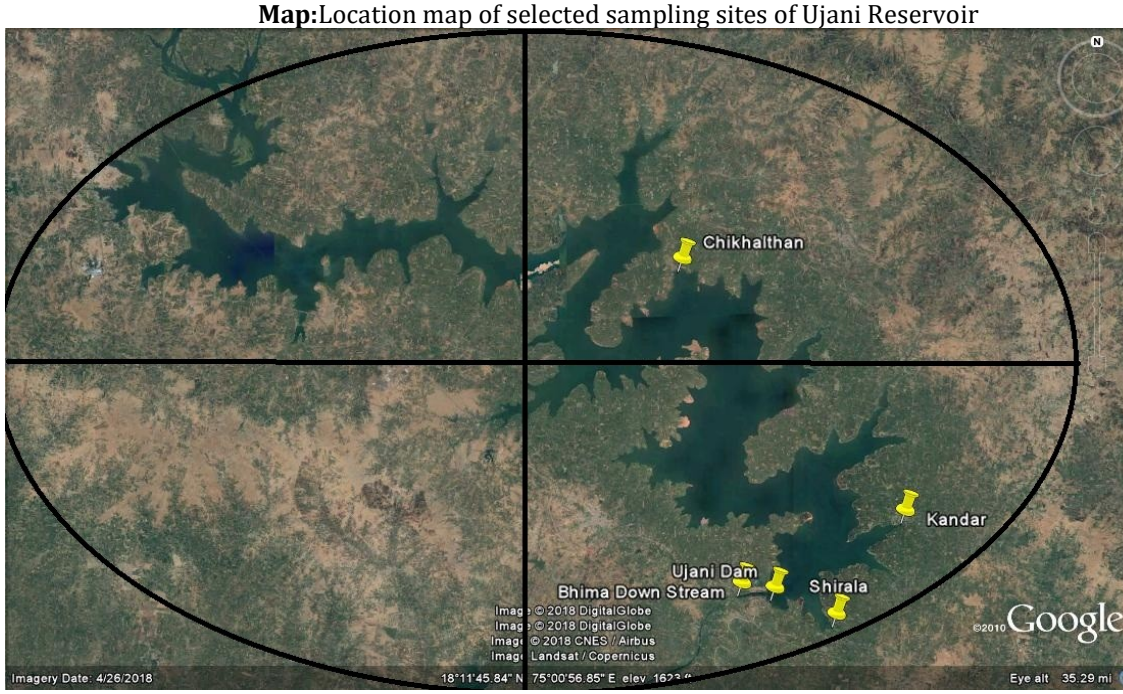
The water quality can be visualized in terms of the physical, chemical & biological properties within which several elements of water quality can be identified. Now in recent days water pollution is due to the alteration in physical, chemical & biological characteristics which may lead to harmful effects on human, other organisms and aquatic biota. Water quality assessment generally involves analysis of physico-chemical, biological and microbial parameters and reflects abiotic and biotic status of ecosystem. (IAAB, 1998; Kulshrestha & Sharma, 2006; Mulani et.al., 2009).

The present investigation regarding Physico-chemical characteristics of Ujani reservoir was carried out from Nov.2014 to Sept.2016. Thirteen parameters were analysed from five different sites, of which, three catchment sites (Shirala, Kandhar and Chikhalthan) along with main Dam site and Bhima river (Downstream). Among five sampling sites, four sites are located towards south- east direction while one sampling site is located towards north- east direction of entire reservoir.

II. MATERIALS AND METHODS

Samples were collected from five selected sampling sites I, II, III, IV and V bimonthly for three seasons i.e. March and May (summer), July and September (rainy), November and January (winter) for two years from Nov.2014 to Sept. 2016. The samples were collected in sterilized bottles using standard procedure (APHA 2012). The methodology used for the determination of different parameters is depicted in following table.

Map: Location map of selected sampling sites of Ujani Reservoir



The geographical co-ordinates of the selected sampling sites are as follows.

Table: 1- Geographical co-ordinates of the selected sampling sites of Ujani reservoir.

Sr. No.	Sampling Site	Geographical Co-ordinates
01	Ujani Dam	N 18°4'6" E 75° 7'24" Alt. 494 m.
02	Bhima River (Down-stream)	N 18°4'21" E 75° 6'15" Alt. 445 m.
03	Shirala	N 18°3'19" E 75° 9'9" Alt. 492 m.
04	Kandhar	N 18°6'32" E 75° 11'27" Alt. 486 m.
05	Chikhalthan	N 18°14'32" E 75° 4'23" Alt. 499 m.

Table 2: Methodology used for analysis of physico-chemical parameters.

Sr.No.	Parameters	Methods used for Analysis
01	Temperature (°C)	Mercury Thermometer (on site)
02	Ph	Hanna Champ pH Meter (on site)
03	Alkalinity (mg/lit)	Determined by methodology of APHA (1998) and Koderkar (1998) (in Lab)
04	Phosphates (mg/lit)	
05	Magnesium (mg/lit)	
06	Chlorides (mg/lit)	
07	Total Nitrates (mg/lit)	
08	Ca Hardness (mg/lit)	
09	Total Hardness (mg/lit)	
10	Total Dissolved Solids (TDS) (mg/lit)	Gravimetric Analysis (in Lab)
11	Dissolved Oxygen (DO) (mg/lit)	Winkler method :Titration (on site)
12	Electrical Conductivity (EC) (mS/cm)	Water Analysis Kit (in Lab)
13	Biochemical Oxygen Demand (BOD) (mg/lit)	BOD Incubator followed by Tritometry (in Lab)

III. RESULTS AND DISCUSSION

Analysis of physico-chemical parameters plays vital role as they influence the distribution of different components of biodiversity. In present investigation, thirteen physico-chemical parameters were analysed with standard procedures as mentioned in methodology.

Water temperature is considered as one of the important physical parameter that controls aquatic life in the reservoir as it directly affects the biochemical reactions. At all the five sampling sites selected, the minimum average water temperature was recorded at site V (Chikhalthan) as 22.00°C during winter and

maximum average temperature was recorded at site III (Shirala) as 28.30°C during summer season.

pH is the measure of the acidity of solution of water. Alkaline water promotes high primary productivity (Kumar and Prabhakar, 2012). The recorded pH at all sampling sites shows minimum values during rainy while maximum values during summer season. The minimum average pH was observed at site IV (Kandhar) as 7.10 and maximum value observed at site I (Ujani Dam) as 8.45.

Electric conductivity (EC) is affected by suspended impurities and amount of ions in water. It reflects mineral salt content of water. The average electrical conductivity was recorded minimum during rainy season and maximum during summer season. The minimum value was recorded at site II (Bhima river- Downstream) as 0.42mS/cm while maximum value was recorded at sampling site V (Chikhalthan) as 0.61mS/cm.

Alkalinity is nothing but the combination of various components in water which leads to increase the pH to alkaline side from neutrality. Average minimum alkalinity was recorded during rainy season at site I (Ujani Dam) as 160.5mg/lit while maximum alkalinity was recorded during summer at site IV (Kandhar) as 202.75mg/lit.

Total dissolved solids (TDS) include ionized and non-ionized reservoir matter which reflects in conductivity. Average TDS in present study was observed in the range of 152.30mg/lit at site II (Bhima river- Downstream) during winter to 317.80mg/lit at site III (Shirala) during summer. So it is clear that, the average TDS is found to be minimum during winter and maximum during summer.

Study of dissolved oxygen (DO) in aquatic ecosystem reflects status of DO balance, in turn reflecting the health of ecosystem. Monitoring the DO levels of water bodies is essential to ensure aerobic conditions and preventing the DO concentration to fall below critical level. The observed DO range in present study was 3.67mg/lit to 6.55mg/lit. The minimum DO value was recorded at site IV (Chikhalthan) during summer while maximum DO value was recorded at site II (Bhima river- Downstream) during rainy season.

Biological/ Bio-chemical oxygen demand (BOD) is an important indicator of overall water quality. Measurement of BOD is very important in the assessment of organic pollution load on aquatic ecosystem. In present investigation, minimum average value of BOD was recorded at site I (Ujani Dam) as 3.39mg/lit during rainy season while maximum average value was recorded at site IV (Kandhar) as 5.97mg/lit during summer.

Phosphates are involved in algal growth. High concentration of phosphates indicates the presence of pollution and is largely responsible for eutrophication. In present study, the minimum average value of phosphate was recorded at site II (Bhima river- Downstream) as 0.18mg/lit during winter season while maximum average value was recorded at site I (Ujani Dam) at site IV (Kandhar) as 1.54mg/ml during summer.

The main sources of Magnesium are minerals and industrial wastes. Beyond permissible limits, it creates harmful effects to aquatic life. The average magnesium values were found to be minimum during rainy season, maximum during summer while relatively less during winter. The minimum value was recorded at site II (Bhima river- Downstream) as 43.47mg/lit and maximum value was recorded at site IV (Kandhar) as 101.03mg/lit.

In fresh water, sources of chlorides are soil and rock formation and waste discharge. High chloride levels may render freshwater unsuitable for agricultural irrigation. In present investigation, minimum average value of chlorides was recorded at site I (Ujani Dam) as 29.54mg/lit during winter season while maximum value was recorded at site IV (Kandhar) as 54.07mg/lit during summer.

The principal sources of nitrates in fresh water are land drainage, plant and animal debris, industrial and municipal wastes, leachates from wastes disposal sites and sanitary landfills. In present investigation, the minimum average values of total nitrates was recorded at site I (Ujani Dam) as 0.67mg/lit during winter while maximum value was recorded at site V (Chikhalthan) as 2.83mg/lit during summer.

Calcium is favourable for flora, fauna of reservoir including fish. High levels of calcium may be beneficial for aquatic biodiversity. The minimum average values of calcium were recorded during rainy season and maximum values were recorded during summer season. The observed range of calcium hardness as 44.15mg/lit at site II (Bhima river- Downstream) to 73.76mg/lit at site V(Chikhalthan).

Total hardness in aquatic ecosystem indicates calcium and magnesium concentrations expressed as mg/lit. Water hardness reflects the nature of the geological formations with which the water has been in contact; this is the reason why surface water is generally softer than ground water. In present study, the minimum average value of total hardness was recorded at site II (Bhima river- Downstream) as 89.12mg/lit during rainy season while maximum value was recorded at site III (Shirala) as 175.70mg/lit during summer season.

Table: 3-Table showing seasonal variations in mean values of various physico-chemical parameters at respective sites I, II, III, IV & V.
(**Abbreviations:** Temp: Temperature, pH: Negative logarithm of hydrogen ion concentration, Ele. Cond.: Electrical Conductivity, TDS: Total Dissolved Solids, DO: Dissolved Oxygen, BOD: Biological/ Biochemical Oxygen Demand, Ca Hard.: Calcium Hardness, Total Hard. Total Hardness)

Sr. No.	Parameters	Site: I			Site: II			Site: III			Site: IV			Site: V		
		Ujani Dam			Bhima River (Downstream)			Shirala			Kandhar			Chikhalthan		
		Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
01	Temp.	26.5	24.5	23.5	25.5	24.0	23.2	28.3	24.4	23.2	26.7	24.4	22.9	28.2	23.9	22.0
02	pH	8.45	7.67	8.07	8.30	7.40	7.87	8.25	7.27	7.85	8.25	7.1	7.77	8.05	7.32	7.72
03	Ele. Cond.	0.58	0.47	0.52	0.55	0.42	0.43	0.59	0.49	0.52	0.53	0.45	0.50	0.61	0.52	0.57
04	Alkalinity	194.0	160.50	175.50	185.50	175.70	180.20	199.25	169.75	182.75	202.75	163.00	183.75	196.75	163.50	178.75
05	TDS	309.19	265.32	189.80	273.99	232.53	152.30	317.80	264.11	208.52	308.78	264.10	188.71	302.49	267.11	195.74
06	DO	5.32	6.45	7.10	6.07	6.55	7.35	5.90	6.47	7.20	3.67	4.45	5.02	5.00	6.22	6.80
07	BOD	4.72	3.39	3.89	4.00	3.55	3.61	5.35	4.02	4.68	5.97	4.50	5.36	5.30	3.83	4.49
08	Phosphates	1.54	0.91	0.45	0.24	0.21	0.18	1.46	1.19	0.36	1.54	1.05	0.76	1.46	1.10	0.78
09	Magnesium	59.20	51.36	54.78	56.58	43.47	49.46	90.63	77.88	78.77	101.03	71.38	82.07	82.44	64.42	72.10
10	Chlorides	47.48	33.88	29.54	41.87	37.16	33.62	50.44	44.22	39.45	54.07	46.01	37.45	48.42	38.47	34.56
11	Nitrates	1.42	0.72	0.67	1.45	1.06	0.70	2.45	2.18	1.72	2.83	1.39	1.35	2.83	1.92	1.19
12	Ca Hard.	67.32	46.57	55.18	57.96	44.15	50.13	71.88	62.35	66.97	69.82	51.00	60.19	73.76	58.59	60.03
13	Total Hard.	126.64	100.94	111.15	116.05	89.12	101.06	175.70	138.55	146.96	174.90	123.50	135.27	157.66	124.14	133.63

IV. CONCLUSION:

Among the five sampling sites analysed, site III (Shirala), site IV (Kandhar) and site V (Chikhalthan) are very close to agricultural land, so these sites are mostly affected by agricultural wastes and fertilizers run-off. These sites are also associated to human population, so these are also affected by waste water discharge including sewage continuously. The water quality index (WQI) of these sites is somewhat poor and water cannot be used for drinking purpose without proper treatment. Sampling sites I (Ujani Dam) and II (Bhima river- Downstream) shows all the physico-chemical parameters within general permissible limits as well as within desirable limits by WHO, so this water can be used for drinking purpose prior to treatment.

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DIVERSITY OF SHORT-HORNED GRASSHOPPERS (ORTHOPTERA: CAELIFERA) FROM DROUGHT PRONE REGION OF SATARA DISTRICT, WITH THE FIRST RECORD OF ACRIDA GIGANTEAN AND ACRIDA TURRITA FROM THE STATE MAHARASHTRA

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ABSTRACT: *Grasshoppers are important producer component of the invertebrate and vertebrate diversity; these are also act as indicator of diversity and healthy ecosystem. In the present study 21 species representing 19 genera belonging to two families and 08 subfamilies have been recorded from various regions of Dahiwadi tehsil, Satara district of Maharashtra. The present exploration is the primary study of orthopteran insects from agricultural lands of Dahiwadi tehsil, Satara district of Maharashtra. Acrida gigantean and Acrida turrita these two species were new report from the state Maharashtra.*

Keywords: *Short horned Grasshoppers,Acrida gigantean, Acrida turrita, Diversity.*

I. Introduction

Order Orthoptera is one of the order of insects which are directly associated with human habitation and includes short horned and long horned grasshoppers, crickets and grouse locusts, ranging from size of 5 mm to 115 mm. Many species of the order produce sound (known as stridulation) by rubbing their wings on wings or wings on legs against each other, the wings or legs contain rows of corrugated tubercles or elevated markings, by rubbing those produce a sound which is mostly unique with respect to species. Grasshoppers are important producer component of the invertebrate and vertebrate diversity; these are also act as indicator of diversity and healthy ecosystem.

A notable taxonomical work on Acrididae was made by Kirby (1914) in the series 'Fauna of British India', Uvarov (1921, 1924, 1927, 1942) studied in detail the Indian Acrididae. Agarwala (1952) give contribution on female copulatory structures in relation to oviposition sites while Roonwal (1956) contributed some studies on the nymphal structures and ecology on Acrididae. Dirsh (1965, 1975), Tandon (1975, 1976), Bhowmik (1985), Shishodia (1987, 1997, 1999), Mandal et. al. 2007. Shishodia et al. (2010), Nayeem & Usmani (2011, 2012), Chandra and Gupta (2012) have worked on the taxonomy as well as on the ecology of this group.

II. MATERIALS AND METHODS

About 157 specimens of grasshoppers were collected from various agricultural areas of Dahiwadi tehsil of Maharashtra. Detailed surveys carried out in various agricultural areas of Dahiwadi tehsil, Satara district of Maharashtra during the period July 2018- October 2018 for the collection of grasshoppers. As they were caught by hand, by forcepsand by the ordinary aerial insect net. The net was used for catching insects individually or by sweeping on grasses, bushes and other vegetables. Collected specimens were preserved dry as well as wet (in 70% Ethanol), phographed. Identification done with the help of Kirby (1914), Mandal et. al. 2007, Shishodia et al. (2010) andNayeem & Usmani (2011, 2012).

III. RESULTS AND DISCUSSION

In the present study 21 species representing 19 genera belonging to two families and 08 subfamilies have been recorded from various regions of Dahiwadi tehsil, Satara district of Maharashtra.

Order: ORTHOPTERA
Suborder: CAELIFERA
Super family: ACRIDOIDEA
Family: ACRIDIDAE
Subfamily: TRUXALINAE

1. *Truxalis indica* (Bolivar, 1902)

Material Examined: 2 ♂3♀Mardi, Satara Dist. 01. viii. 2018; 3♀, Jashi, Satara Dist. 16. viii. 2018.
Distribution: India: Andhra Pradesh, Bihar Chhattisgarh, Gujarat, Madhya Pradesh, Maharashtra, Orissa,

Karnataka and Tamil Nadu. **Elsewhere:** Myanmar and Sri Lanka.
Remark: Common medium to large sized species found in all type grassy vegetation, colour morphs observed.

Subfamily: ACRIDINAE
Genus: *Acrida* Linnaeus, 1758
2. *Acrida exaltata* (Walker, 1859)

Material Examined: 1♂ 2♀,Dahiwadi, Satara Dist. 04.viii.2018; 1♀ , Mardi, Satara Dist., 12. viii.2018.
Distribution: India: (Widely distributed). **Elsewhere:** Afghanistan, Arabia, Bangladesh, Iran, Nepal, Pakistan, Saudi Arabia, Sri Lanka, Tibet, Yemen & West Eden.
Remark: Common medium to large sized species, mostly found in live green grassy vegetation.

3. *Acrida gigantea* (Herbst, 1786)
Material Examined: 1♂ 1♀,Dahiwadi, Satara Dist. 04.viii.2018.
Distribution: India: Himachal Pradesh, Madhya Pradesh, Tamil Nadu and Utlarakhand. **Elsewhere:** Africa and Nepal and Pakistan.
Remark: Quite rare medium sized species, mostly found in agricultural lands vegetation. First report from Maharashtra state.

4. *Acrida turrita* (Linnaeus, 1758)
Material Examined: 1♀ Dahiwadi Satara Dist, 18.viii.2018; 2♂ 2♀ Jashi,Satara Dist, 19.viii.2018.
Distribution: India: Himachal Pradesh. **Elsewhere:** Africa, Asia, Pakistan, South Europe.
Remark: Quite rare medium to large sized species, mostly found in agricultural lands vegetation. First report from Maharashtra state.

Genus: *Phlaeoba* Stal, 1860
5. *Phlaeoba antennata* Brunner, 1893

Material Examined: 1♀ , Jashi. Satara Dist, 11. viii. 2018, 2♂Mardi, Satara Dist, 10. viii. 2018.
Distribution: India: Arunachal Pradesh, Assam, Kerala, Maharashtra, Orissa, Rajasthan and West Bengal. **Elsewhere:** Bangladesh, Borneo, Myanmar, China, Malaysia, Tonking and Sumatra.
Remark: This species generally occurred in thick vegetation.

Subfamily OEDIPODINAE
Genus *Gastrimargus* Saussure, 1884
6. *Gastrimargus africanus africanus* Saussure, 1888

Material Examined: 1♂ 2♀Dahiwadi,Satara Dist.,17.viii.2018, 2♂ 2♀ Mardi, Satara Dist , 13.viii.2015.
Distribution: All over India **Elsewhere:** Africa, Myanmar, Nepal, Pakistan, Saudi Arabia, Sri Lanka, Thailand, Tibet and Yemen.
Remark: This species generally occurred in barren lands, and behind agricultural lands.

Genus *Trilophidia* Stal, 1873
7. *Trilophidia annulata* Thunberg, 1815

Material Examined: 1♀ Dahiwadi Satara Dist, 18.viii.2018; 2♂ 2♀ Jashi,Satara Dist, 19.viii.2018.
Distribution: All over India **Elsewhere:** Afghanistan, Bangladesh, Borneo, China, Hong Kong, Japan, Java, Korea, Malaysia, Mongolia, Myanmar, Nepal, Pakistan, Philippines, Sarawak, Singapore, Sri Lanka, Sumatra, Taiwan, Thailand and Vietnam.
Remark: This species generally occurred in barren lands.

Genus *Dittopternis* Saussure, 1884
8. *Dittopternis venusta* (Walker 1870)

Material Examined: 1 ♂ 2♀ Mardi, Satara, Dist, 07.viii.2018; 2♀ Dahiwadi, Satara Dist, 15.viii.2018.
Distribution: India: Andhra Pradesh, Chhattisgarh, Karnataka, Madhya Pradesh, Manipur, Meghalaya, Maharashtra, Orissa, Tamil Nadu, Tripura and West Bengal. **Elsewhere:** Sri Lanka
Remark: It is associated with small grasses.

Genus *Aiolopus* Fieber, 1853
9. *Aiolopus thalassinus tamulus* (Fabricius, 1798)

Material Examined: 2 ♂ 1 ♀ Mardi, Satara, Dist.19.viii.2018, 1♂ Jashi, Satara Dist. 20. viii. 2018.
Distribution: All over India **Elsewhere:** Australia, Bangladesh, Borneo, Brunei, Celebes, China, Hong Kong, Japan, Java, Malaysia, Myanmar, New Guinea, Pakistan, Papua, Philippines, Singapore, Sri Lanka, Sumatra, Taiwan, Thailand and Timor.
Remark: This species is associated grass and attached on cultivated field.

Genus *Oedaleus* Fieber, 1853

10. *Oedaleus senegalensis* (Krauss, 1877)

Material Examined: 1♂ 1♀, Dahiwadi, Satara Dist, 20. viii. 2018.

Distribution: India: Andhra Pradesh, Bihar, Delhi, Jammu and Kashmir, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttarakhand & West Bengal. **Elsewhere:** Afghanistan, North Africa, Pakistan & Western U.S.S.R.

Remark: This species is associated with barren lands and agricultural lands.

Genus *Morphacris* Walker, 1870

11. *Morphacris spp.*

Material Examined: 2 ♂ 1♀, Jashi, Satara, Dist. 24. viii. 2018.

Remark: This species is associated with barren lands and agricultural lands.

Subfamily OXYINAE

Genus *Oxya*, Serville, 1831

12. *Oxya spp.*

Material examined: 2 ♂ 1♀, Jashi, Satara, Dist. 24. viii. 2018.

Remark: This species is found on small grass and bushy zone adjoining water ponds. It damages the seedlings of growing crops.

Subfamily CYRTACANTHACRIDINAE

Genus *Cyrtacanthacris* Walker, 1870

13. *Cyrtacanthacris tataricatatarica* (Linnaeus, 1758)

Material Examined: 2♂, Mardi,Satara, Dist, 26. viii. 2018; 1 ♀, Mardi,Satara Dist, 23. viii. 2018.

Distribution: Most parts of India **Elsewhere:** Africa, Bangladesh, Central America, Hainan, Indonesia, Madagascar, Mediterranean Region, Myanmar, Nepal, Pakistan, Philippines, Red-Sea, Sahara, Saudi Arabia, Seychelles, Sri Lanka, South West Asia, Sumatra and Thailand.

Remark: It is found feeding on wild and cultivated plants. This species occurs in plains as well as in hilly regions.

Genus *Patanga* Uvarov, 1923

14. *Patanga succincta* Johansson 1763

Material Examined: 1♂, Jashi, Satara, Dist, 20. viii. 2018; 1 ♀3♂Mardi, Satara, Dist, 30.viii. 2018.

Distribution: Most of India **Elsewhere:** Australia, Borneo, China, Hainan Island, Japan, Malaysia, Myanmar, Pakistan, Philippines, South Arabia, Sri Lanka, South East Asia, Sumatra and Taiwan.

Remark: It is commonly called as Bombay locust. Adults and Nymph feed on a variety of plants and found in plains as well as in agricultural lands.

Genus *Xenocatantops* Dirsh & Uvarov, 1953

15. *Xenocatantops humilis humilis* (Serville 1838)

Material Examined: 2♂, Dahiwadi, Satara, Dist, 30.viii.2018; 1♀, Jashi, Satara, Dist, 22.viii. 2018.

Distribution: Most parts of India **Elsewhere:** Bangladesh, Borneo, Indo-China, Java, Lombok, Malaysia, Myanmar, Nepal, New Guinea, Philippines, Sumatra, Sri Lanka, Thailand, Tibet, Vietnam and Yunnan.

Remark: This species is generally found in agricultural lands, a heavy infestation of this species is found in new cultivated fields.

Subfamily GOMPHOCERINAE

Genus *Chorthippus* Fieber, 1852

16. *Chorthippus spp*.

Material Examined: 2 ♂1 ♀, Mardi, Satara Dist, 06. ix. 2018.

Remark: This species is generally found in agricultural lands but in very few in number.

Genus *Choroedocus* Bolivar, 1914

17. *Choroedocus robustus* (Serville, 1839)

Material Examined: 1♂, Dahiwadi, satara Dist, 09. ix. 2018.

Distribution: India: Arunachal Pradesh, Andhra Pradesh, Maharashtra and Assam. **Elsewhere :** Bangladesh.

Remark: This species occurs amongst mixed vegetation zone and found on the agricultural area. Both Nymphs and adults are abundant from the months April to August.

Subfamily TROPIDOPOLINAE

Genus *Tristria* Stal, 1873

18.*Tristria pulvinata* Uvarov, 1921

Material Examined: 3 ♂, Mardi, Satara Dist, 13. ix. 2018.

Distribution: India: Andhra Pradesh, Assam, Bihar, Delhi, Haryana, Karnataka, Kerala, Maharashtra, Meghalaya, Orissa, Punjab, Tamil Nadu, Uttarakhand, Uttar Pradesh and West Bengal. **Elsewhere:** Sri Lanka.
Remark: This species found on various grasses.

Superfamily: PYRGOMORPOIDEA
Family: PYRGOMORPHIDAE
Subfamily: PYRGOMORPHINAE
Genus *Atractomorpha* Saussure, 1862
19. *Atractomorpha crenulata* Fabricius, 1793

Material Examined: 2 ♂ 2♀ Dahiwadi, Satara Dist, 16. ix. 2018; 2 ♀ Jashi, Satara Dist, 08. ix. 2018.
Distribution: All India **Elsewhere:** Bangladesh, Cambodia, Lasso, Maldives Island Malaya, Myanmar, Nepal, Pakistan, Sri Lanka, Sumatra, South Vietnam and Thailand.
Remark: This species associated with a small grasses in the agricultural lands.

Genus *Chrotogonus* Serville, 1838
20. *Chrotogonus (Chrotogonus) trachypterus trachypterus* Blanchard 1836

Material examined: 2 ♂ 2♀ Dahiwadi, Satara Dist, 29. ix. 2018; 2 ♀ Mardi, Satara Dist, 03. ix. 2018.
Distribution: Most parts of India.
Remark: This species is found on low grass and shrub and associated with a good deal of bare ground.

Genus: *Coptotettix* Bolivar, 1887
21. *Coptotettix conspersus* Hancock, 1915

Material Examined: 2♂ 2♀ Mardi, Satara Dist, 30.ix.2018; Jashi, Satara Dist, 18. x. 2018.
Distribution: India: Arunachal Pradesh, Assam, Chhattisgarh, Himachal Pradesh, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Orissa, Tripura, Uttarakahand, Uttar Pradesh and West Bengal.
Remark: This species is found on barren lands and also in agricultural lands.

The present exploration is the primary study of Orthopteran insects from agricultural lands of Dahiwadi tehsil, Satara district of Maharashtra. In the short period enlisting 21 species under 19 genera of short horn grasshoppers is significant and indicates continuous drought prone region is also having a significant diversity.*Acrida gigantean* and *Acrida turrita*, these two species were new report from the state Maharashtra, this indicates that in the study region there is possibilities of different diversity is present which is till not recorded from the region.

V. ACKNOWLEDGMENT

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***Truxalis indica* (Bolivar, 1902)**

***Acrida exaltata* (Walker, 1859)**

***Acrida turrita* (Linnaeus, 1758)**

***Acrida gigantea* (Herbst, 1794)**

***Patanga succincta* Johansson 1763**

***Gastrimargus africanus africanus* Saussure, 1888**

***Choroedocus robustus* (Serville, 1839)**

***Chrotogonus (Chrotogonus) trachypterus trachypterus* Blanchard 1836**

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Trilophidia annulata Thunberg, 1815

Chorthippus spp.

Xenocatantops humilis humilis (Serville 1838)

ADVERSE DRUG REACTIONS IN TUBERCULOSIS PATIENTS DURING MDR TREATMENT IN SATARA DISTRICT

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ABSTRACT: *Multidrug resistant tuberculosis (MDRTB) poses difficulties in diagnosis and treatment, including increase frequency of adverse drug reactions to antituberculosis drugs. The main reason for non-adherence to MDR-TB is Adverse drug reaction. The main aim of the study was to illuminate the profile of adverse drug reactions (ADRs) associated with second line anti-tubercular treatment for drug resistant tuberculosis in Satara District. The study included 100 diagnosed MDR-TB patients for period of two years. Adverse reactions were categorised, observed and noted as per the patients. Every patient was followed up during treatment.100 patients were selected for study, every patients was showing mild and moderate adverse effect, such as Gastrointestinal symptom, nausea, vomitting, giddiness, occular toxicity, renal toxicity, anthralgia, cutaneousreaction,hepatitis,neurological disorders, psychiatric disturbances and hypothyroidism. Some drugs were terminated for some period in some cases due to adverse reaction. Early detection and management is most important in drug resistant tuberculosis. Incidence of adverse effect is more than 90% in multi drug resistant . This highlighted the importance to develop good strategies to improve the quality of patient care and to control MDR-TB safety.*

Keywords: *Adverse drug reaction, anti-TB therapy, multi drug resistant TB, drugs, tuberculosis.*

I. INTRODUCTION

The emergence of resistance to drugs used to treat tuberculosis (TB), and particularly multidrug-resistant TB (MDR-TB), has become a significant public health problem in a number of countries and an obstacle to effective TB control.Multi-drug resistant TB i.e. MDR-TB is defined as *Mycobacterium tuberculosis*resistant to isoniazid and rifampicin with or without resistance to other drugs (RNTCP Guidelines 2012). MDR-TB is a man made phenomenon. Because of poor treatment, poor drugs and poor adherence this lead to the development of MDR-TB. An inadequate or poorly administered treatment regimen allows drug resistant mutants to become the dominant strain in a patient infected with TB. There are 27% cases of MDR-TB in India and out of that 20% cases are in Maharashtra. Mortality rate is high in MDR-TB. Treatment of MDR TB requires the use of expensive and toxic second-line anti-tubercular drugs which are given for a longer duration(Iseman MD1993). The Revised National Tuberculosis Control Program (RNTCP) in India follows the internationally recommended directly observed treatments (DOTS) plus guidelines for treatment of MDR-TB known as Category IV (CAT IV) regimen from August 2007 onward. Poor management of adverse effects increases the risk of default or irregular adherence to treatment and may result in death or permanent morbidity.Despite the positive therapeutic effects, studies have shown that utilization of multi drug regimen cause undesirable adverse drug reactions (ADRs) of various kind in body such as gastrointestinal (GI) disorders, Giddiness,Occular toxicity,Renal toxicity,Anthralgia, Neurological disorders, etc.(Yee D.*et al* 2003 andChhetri A.*et al* 2008). Studies suggest that all anti-tubercular drugs develop ADRs(Dhingra VK *et al* 2004 and Chukanov VI *et al*2004). Adverse reaction are not life threatning but only they cause non-adherence to treatment. Many study has been done on Tuberculosis patient one of the study was done in Satara District to know the effectiveness of DOTs therapy in TB patient (Bhatia P*. et al*2014*)*

II. MATERIALS AND METHODS

This study has limited scope and limits only to the juridiction of Satara District. This study was carried out in the Department of District Tuberculosis Center in Civil Hospital, Satara, from 2015 to 2017. For study patients were randomly selected having multi-drug resistant TB. The study included 100 consecutive diagnosed MDR-TB patients.The patients were selected irrespective of age,sex and race. For study monthly follow-up was taken.During the assessment patient showing ADR to the second line anti-TB drugs was recorded when the attendingphysician confirmed the ADR and also laboratory screening is done. These ADRs were recognized, categorized and then analyzed as per severity. Severity of the ADRs were classified according to MDR-TB guidelines by Central TB Division (May 2012).Minor adverse reactions are extremely common in MDR-TB but if show serious adverse reaction he/she is ideally admitted to District TB center.

Regimen for MDR-TB-

Regimen comprises 6 drugs- Kanamycin, Levofloxacin, Ethionamide, Pyrazinamide, Ethambutol and cycloserine for 6-9 months of the Intensive phase and 4 drugs- Levofloxacin, Ethionamide, Etambutol and cycloserine for the 18 months of continuation phase(RNTCP Guidelines 2012).

Reactions due to drugs are as follows:

1. Gastro-intestinal symptoms-
   Causes nausea and vomitting due to many drugs such as ethinoamide, PAS, Pyrazinamide and Ethambutol. Patient who complain about this are advised to take drugs with banana.
2. Giddiness-
   It could be due to Aminoglycosides, Ethionamide, Fluroquinolone or Pyrazinamide. Patient who complain about this offending drug is indentified by giving the drugs individually and observing he response.
3. Occular toxicity-
   Patient who complain about burning of vision or disturbance in colour vision. Ethambutol is withheld and opthalmolofist is reffered for opinion.
4. Renal toxicity-
   Patient who complain about this then all drugs are withheld and renal function test is done and if required opinion of nephrologist is taken.Common offending drug is an aminoglycoside.
5. Anthralgia-
   The offending drugs are Pyrazinamide and fluroquinolone. Patient who complain about this are prescribed to take Paracetamol 500mg three times a day or aspirin 300mg three times a day. If no improvement is seen then dosage of Pyrazinamide or Levofloxacin are reduced or the drug withheld temporarily.
6. Cutaneous reaction-
   Hypersensitivity reactions occur with any drugs used and are commonly managed with anti-histamines. If severe reaction occur and do not respond to anti-histamine individual drug is identified and then terminated.
7. Hepatitis-
   This could be due to the combined effect of potentially hepatotoxic drugs such as Pyrazinamide and Ethionamide.
8. Neurological symptoms-Peripheral neuropathy
   The common offending drugs are Cycloserine and Ethionamide. To prevent occurrence of such adverse reaction, all patients on an RNTCP Regimen for MDR-TB receive daily Pyridoxine 100mg is given.
9. Psychiatric Disturbance-
   The common offending drugs are Cycloserine, Fluroquinolone or Ethionamide. In cases of suicidal tendencies and other psychiatric disturbances, the first offending drug is Cycloserine, followed by Ethionanide and Fluroquinolone. These drugs are withheld and further management of the patient is done in consultation.

Role of District TB Center in the management of ADR-

Whenever a patient has serious adverse reactions to any of the second line anti-TB drugs the patient is admitted at the district TB center and the committee decides on further management of the patient.This may require to hold or discontinue the medicines of treatment.Ancillary drugs are also available for the management of adverse reactions for the patient free of cost.

There are various drugs available to manage Adverse drug reaction are as follows:

ADRs	Drugs to manage **ADRs**
Nausea, vomiting, upset Stomach	Metoclopramide, dimenhydrinate, prochlorperazine, promethazine, bismuth subsalicylate, donperidone
Heartburn, acid indigestion, sour stomach, ulcer	H2-blockers (ranitidine, cimetidine, famotidine, etc.), Proton pump inhibitors (omeprazole, lansoprazole, etc.) Avoid antacids because they can decrease absorption of flouroquinolone eg. alumnium hydroxide

Diarrhoea	Loperamide
Depression	Selective serotonin reuptake inhibitors (fluoxetine, sertraline), tricyclic antidepressants (amitriptyline)
Severe anxiety	Lorazepam, diazepam, clonazepam
Insomnia	Any hypnotic
Prophylaxis of neurological complications of cycloserine	Pyridoxine (vitamin B6)
Anrthralgia	Ibuprofen, paracetamol,codeine,diclofenac
Cutaneous reactions, itching	Hydrocortisone cream, calamine, caladryl lotions
Systemic hypersensitivity Reactions	Antihistamines (diphenhydramine, chlorpheniramine, dimenhydrinate), corticosteroids (prednisone, dexamethasone)

CASE STUDY

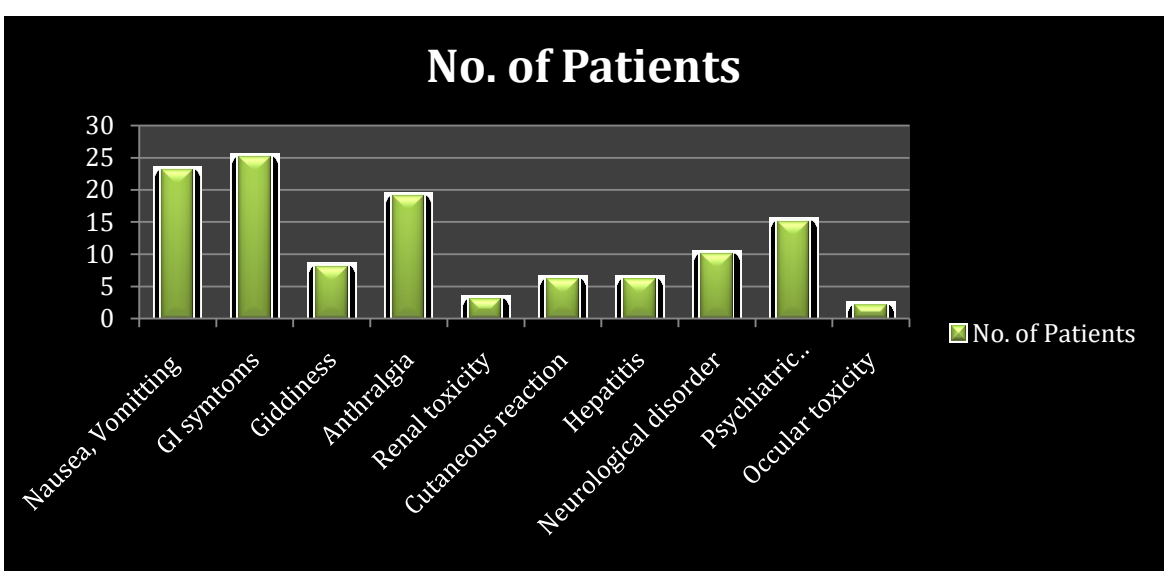
III. OBSERVATION-

Total cases studied 100

During the 24 month period of MDR-TB treatment total 100 patients were selected for the study. Among these no patients were transferred out or died during monitoring. The male number was high than females taking MDR-TB drugs. The Adverse drug reaction was recorded as the patient was coming for follow-up. With the help of physician, DOT provider and nurse drug reaction was recorded.

100 patients were showing these Adverse reactions to MDR-TB drugs,

Nausea, Vomitting	GI Symptoms	Giddiness	Anthralgia	Renal Toxicity	Cutaneous Reaction	Hepatitis	Neurological Disorder	Psychiatric Disturbane	Occular Toxicity
23	8	8	19	3	6	6	10	15	2



IV. RESULTS

Out of 100 patients, many patients showed one or more ADR. 23% patient suffered from nausea and vomitting, 25% patient shows Gastro-intestinal symptoms. 8% Giddiness, 19% Anthralgia,6% Hepatitis, Neurological disorder 10%, Psychiatric disturbance 15%, Ocular toxicity and renal toxicity were the least common 2 and 3%.

V. DISCUSSION

The present study was undertaken to find out the ADRs of MDR-TB drugs in MDR-TB patients in District TB Center, Satara.During study we understood that drug reactions are more in males than female. It may be due to fact that males are having higher risk factors like smoking, alcoholism, and drug addiction to get TB (Lonnroth K,*et al* 2008). In the present study majority of MDR patient suffer more due to MDR-TB because of regimen and the adverse reactions which occur due to drugs of MDR. The most common ADRs were GI symptoms (25%). The drugs which are responsible for these side effects are ethinoamide, PAS, Pyrazinamide and Ethambutol. 6% patients developed skin reactions this side effect may be due to PZA, RFP and INH(Sinha *et al* 2018). Female cases are more affected by ADRs when compared with male. In general female are at higher risk of ADR(Puavilai S. *et al* 1989). It can be because they pass through pregnancy, Menstrual cycle, etc, which modify the drug response(Wilson K. !984). Studies conducted in UK and Canada also reported to have higher incidence of ADR in female than males. (Yee D, *et al* 2003).The present study suggest that when proper management of ADR is taken then patient can be cure and drugs if terminated due to ADR then MDR patient will be defaulter so to cure the MDR proper management of ADR is very important. Because of this patient will not enter in XDR phase and will cure in MDR.

VI. CONCLUSION

The study shows that every anti-tubercular drugs has side effects. But by careful observation and laboratory screening drug reactions can be identified properly and patient can be cured as early as possible. Awareness is very important in Tuberculosis. If patient will know about drug reaction he/she can contact the DOT provider or physician and treatment will not stop and there will be decreased in non-adherence to treatment and patient will cure 100% and will not enter in XDR phase of Tuberculosis.

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EFFECT ON AVIFAUNA DUE TO DUMPED WASTE MATERIAL ITIPPHALLI RESERVIOR, JAT, DIST-SANGLI, (M.S.)

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ABSTRACT: *Tipphalli reservoir is located at 4.7 K.m north at the outskirt of jath tahasil. The reservior is situated latitude 17.08135and longitude 75.191820. The water of reservior is used for various purposes such as drinking, washing, bathing, agriculture and fishing. Tipphalli reservior shows diversity in fauna and weeds.*
The wetlands having abundant food availability that harbors variety of bird's species. Due to human anthropogenic activities such as washing, bathing and dumping waste material like non- degradable of plastic bags, plastic bottles, waste flowers, the emission of lord Ganesh idols ,waste clothes, unwanted net material or gears. Due to dumped materialthe avifaunal diversity was affected in the study area .The study was conducted during March 2016 to February 2018, this paper provides an overview of status of birds and there occurrence in the study area.

Keywords: *Tipphalli, harbors, anthropogenic, non-degradable, emission*

I. INTRODUCTION:

India being a megadiversitycenterharbors about 1200 species of birds that amount to 13% of total birds of the world [Ali,2012].Water bodies and wetlands help mankind in ways, such as agriculture, fishing, sewage disposal, grazing, storage of groundwater; tourism; birding*etc.*[P.V.Darekar.*et.al.*2018]. Birds play important role in the ecosystem as potential, pollinators, scavengers, and bio-monitors in controlling insect's pests& also as excellent ecological indicator [Suresh .M.Kumbar .*et.al.*]Diversity of the avifauna is one of the evaluate quality of habitats [Puri S.D. & Virani R.S.].Nowadays, avifaunal diversity has been decreasing due to the destruction of natural habitats and human disturbance [Bhadja &Vaghela, 2013]. The geographic location of a wetland may determine how and when birds will use it or use adjacent habitat [Manikannan,2011].

II. MATERIAL AND METHODS:

Materials:

The present investigation is carried on a water body,Tipphalli reservior is located at 4.7 K.m north at the outskirt of jath tahasil. The reservior is situated latitude 17.08135and longitude 75.191820. Tipphalli reservoir which is located in a draught prone tahasil of Sangli district. The area is surrounded by agricultural land. The surrounding area of reservoir is rich in a varieties of woody vegitation **i**ncludes lemon, accassia, ziziphus, neem, tamarinds *etc*. Many fruit culture land is close to this water reservoir. The reservoir is attractive place for good habitat of varieties of birds.

For the close behavior of avifauna the binocular (Nikon Aculon A211) is used. For the close photography and the digital camera different zoom lenses was used.(Nikon-3200 with lens – 50 -150 mm and 70-300mm and Nikon P900). Birds were identified on the field using guides by Ali [1]

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Google map showing the location of study site.

Methods:

The regular monitoring for the study the habitat of bird and the human anthological activities for the period of 24 months. I.e. March, 2016 to February, 2018. A conclusion regarding the effect of human interference on bird's habitat is drawn. Comprehensive literature review of published studies on plastic ingestion by water birds and more general diet studies we used online database and evaluated all studies that were published from 1950 to 2018.

III. RESULT AND DISCUSSION:

Jath tahasil is draught prone tahasil of Sangli district. Even though the tahasil is dotted is dotted with many minor and major irrigation projects. These reservoirs are constructed for the irrigation and drinking purposes. Tipphalli reservoirs located at the latitudeat 4.7 K.m north at the outskirt of jath tahasil. The reservior is situated latitude 17.08135and longitude 75.191820, the water is used for washing, bathing, drinking, agricultural as well as for fishing.

The area is surrounded by agricultural land. The surrounding area of reservoir is rich in a varieties of woody vegitation includes lemon, julifera, accassia, ziziphus, neem, tamarinds *etc.*Fruit culture farming is also practicing from farmers at the bank of Birnal reservoir land is close to this water reservoir. The reservoir is attractive place for good habitat of varieties of birds.

Due to abundant food availability, the reservoir noted local; local migratory, winter migratory avifauna. As the reservoir surrounded by an agricultural field, an isolated human population the avifauna habitat was destructed. The Tipphalli reservoir is disturbing from human anthropogenic activities by dumping of wastes such as used plastic products waste clothes, Lord Ganesha idols, waste flowers and garlands *etc.*Washing of clothes and vehicles, utensils, domestic animals and use of detergents (Soap and shampoo) while bathing and swimming is regular activities of villagers.


Vehicles washing in reservoir


Washing clothes in reservoir

The Tipphalli reservoir is on the attack of sever pollution. Because of such disturbances the avifauna is affected. Due to chemical content, the fish number was decreased due to which it affected on piscivorous birds.

Birds are integrated with farmer also by extensive application of chemical fertilizers and pesticides regularly. This affects the bird's population through unavailability of its regular prey and through the harmful effect of chemicals [Blus & Henny, 1997].

Dumped Plastic material

plastic bottle

The plastic bottles, toys, bags discarded fishing nets *etc* were observed nearby reservoir. Plastic contains a variety of toxic additives including colorants, plasticizers and heat and violet stabilizer [Gregory1978]. As the plastic ingestion can cause painful death in aquatic birds, A comprehensive review of plastic ingestion by marine birds has been provided byStranded birds may have higher than average plastic loads because the ingested plastic has affected the birds are less discriminating and eat more plastic immediately prior to stranding [Bourn &Imber 1982;Ryan 1987].

Waste material near Tipphalli reservoir

God Ganapati idol to immerse

The waste clothes were also found dumped in reservoir, which affects the avifauna. Threads and fibres may result in obstruction more frequently than other debris types because they form dense, intertwined balls in gizzard blocking the entrance to the intestine [Parslow & Jefferies 1972; Day et al.1985].

IV. CONCLUSION:

The study site was affected by anthropogenic activities, but now a day's people are aware of the environment, water pollution causes, Due to the unwanted human activities this water reservoir is under threat of loss. This water reservoir should be free from human astrological activities.

Suggestions:

I. A proper monitoring and a conservation strategy should be implemented by irrigation and forest department.

II. Non government organization (NGO)and youth associations of village should take proper steps to keep this water body free from pollution.
III. There is need to remove of dumped waste material from reservoir.
IV. The conservation of flora and fauna around the reservoir should take by villagers.
V. More plantations should be done around the reservoir.

V. ACKNOWLEDGMENT

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BLATTODEA FAUNA OF DROUGHT-STRICKEN REGION OF SATARA DISTRICT OF MAHARASHTRA

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ABSTRACT: *Cockroaches have medical importance as they transmit pathogens which born diseases and as they are omnivorous insects at as manipulators. In all over study 7 species observed in which the heavy infestation observed by Blattella germanica in domestic as well as in agriculture lands followed by Periplaneta Americana. Blattella humbertiana, in agricultural lands and Supella (Supella) longipalpa in domestic region found in satisfactory number. Pycnoscelus surinamensis, Blatta orientalis, Neostylopyaga rhombifolia found in very less number. The record of 7 identified and 2 unidentified species from the drought prone region of Satara district proven that the dry region of Satara district also have a satisfactory diversity.*

Keywords: *Blattodea, Drought stricken region, Pests, Diversity.*

I. INTRODUCTION

Cockroaches are commonly found around human habitation as they get there food and shelter. They are with various sizes small to medium, cursorial, omnivorous insects and some species are serious pest of agriculture. They are generally nocturnal, but some species related to the tree and bush which are diurnal in habit. Body is dorsoventrally flattened; head is somewhat triangular, enlarged triangular plate like pronotum, body not much elongated and broad abdomen. These insects have medical importance as they transmit pathogens which born diseases and as they are omnivorous insects they act as manipulator of forest and domestic wrests by supplying decomposing bacteria through fecal matter. The comprehensive work to enhance the knowledge on Indian Blattodea by Kirby (1904), Shelford (1906), Hanitsch (1915), Bruüning (1948), Rehn (1951), Princis (1966, 1971), Mukherjee (1989, 1993), Roth (1996), Mandal (1995, 2006, 2007), Mandal et al. (2000) and Prabakaran et al. (2009), Jadhav & Sharma (2012), Gaikwad et al. (2014) and (Gaikwad et al. 2015).

From the worldwide 4,000 known species belongs to the 6 families (Roth 1999, 2003) under 445 genera. From the India 156 species reported under 57 genera in 5 families (Mandal 1995, 2000). The blatted fauna of Maharashtra (Jadhav & Sharma 2012) represents 12 species belonging to 10 genera under 4 families. However, earlier *Rhicnoda natrix* and *Rhicnoda rugosa* (Nesemann et al. 2010), *Supella* (*Supella*) *longipalpa* (Gaikwad et al. 2014) and *Hemithyrsocera palliata* (Gaikwad et al. 2015) was recorded Maharashtra which are not included in the fauna of Maharashtra (Jadhav & Sharma 2012). The total 16 species belonging to 13 genera of Blattodea has recorded from Maharashtra till now.

The specimens of Blattodea as they are mostly nocturnal were collected during evening hours 4 pm to 6 pm using insect sweeping net and in night hrs (7 pm to 11 pm) during June 2018- October 2018) with the help of the torch and at light sources by hand picking method. They were killed and preserved as wet preservation method. They were identified with the help of literature of Prabhakaran (2010),Jadhav and Sharma (2012) and Gaikwad et al. (2014).

In the present study, 7 species in all are reported representing 6 genera under 3 families and 4 subfamilies, reported from the some parts of Satara districts. In these three familie, the Blattellidae and Blatidae family comprises three species each and family Blaberidae comprises one family. All over four subfamilies reported, subfamily Blattinae is the dominant with 3 species followed by subfamily Blattellinae represents 2 species, while subfamily Pseudophyllodromiinae and Epilamprinae are represented by 1 species, respectively. The species reported in this region are briefly diagnosed. The information on taxonomy and distribution of these species is scanty; hence the species reported from this region are described with photographs and distribution records.

Systematic checklist
Family: Blattellidae
Subfamily: Blattellinae

1. *Blattella germanica* (Linnaeus, 1767)
2. *Blatella humbertiana* (Saussure, 1863)

Subfamily: Pseudophyllodromiinae

3. *Supella* (*Supella*) *longipalpa* (Fabricius, 1798)

Family: Blaberidae
Subfamily: Epilamprinae

4. *Pycnoscelus surinamensis* (Linnaeus, 1758)

Family: Blattidae
Subfamily: Blattinae

5. *Blatta orientalis* Linnaeus, 1758
6. *Neostylopyaga rhombifolia* (Stål, 1813)
7. *Periplaneta americana* (Linnaeus, 1758)

Systematic account
Order: Blatiodea
Family: Blatiellidae
Subfamily: Blatiellinae
Genus: *Blattella* Caudal, 1903

1. *Blattella germanica* (Linnaeus, 1767)

1767. *Blatta germanica* Linnaeus, Syst. Naturae 1(2) (ed.12) : 688.
Material examined: 2 ♂3♀,Mardi, Satara Dist. 01. viii. 2018; 3♀, Jashi, Satara Dist. 16. viii. 2018.
Diagnosis: Small in size. Head with vertex exposed. Pronotum transeverse, gradually rounded laterally. Tegmen relatively elongated, the subcosta is shorter than anal field, discoidal vein forked before median point. 2 to7 abdominal tergites with latero-caudal portion produced as lobes, 7 and 8 tergites narrowly visible. Supra anal plate semi circular or subtriangular, with lateral margin moderately convergent, weakly convex at the posterior region. Subgenital plate with posterior margin forming a large broad rounded lobe.
Distribution: This species widely distributed species in the India.
Remark: Common medium to large sized species found in all type grassy vegetation, agricultural lands and human vegetation.

2. *Blattella humbertiana* (Saussure, 1863)

1863. *Polyzosteria humbertiana* Saussure, Mem. Soc. Geneve, 17: 131.
Material Examined: 1♂ 2♀ Dahiwadi, Satara Dist. 04.viii.2018; 1♀, , Mardi, Satara Dist., 12. viii.2018.
Diagnosis: Brown in colour, head yellow, frontal region yellowish brown. Small in size. Head with vertex exposed. Cerci slender. Supra anal plate strongly transverse, with distal margin broadly convex. Subgenital plate symmetrical. Female plate simple, free margin broadly convex but suddenly and distinctly concave below cerci.
Distribution: India: Karnataka, Meghalaya, West Bengal, Tripura and Arunachal Pradesh.
Remark: Common medium sized species, mostly found in live green grassy vegetation and eventually seen around the light source.

Subfamily: Pseudophyllodromiinae
Genus: *Supella* Shelford, 1911

3. *Supella* (*Supella*) *longipalpa* (Fabricius, 1798)

1798. *Blatta longipalpa* Fabricius, Suppl. Ent. Syst. Rafniae., 185.
Material Examined: 1♀ Dahiwadi Satara Dist, 18.viii.2018; 2♂ 2♀ Jashi,Satara Dist, 19.viii.2018.
Diagnosis: Pronotum blackish brown with broad yellowish lateral margin; tegmina yellow with a large reddish brown basal spot and also a small oblique paler band. It is winged of a brown colour with varied dark markings. Generally colour light brown. Sexes are dissimilar. Near the apex of the anal fields a broad pale coloured band crosses the tegmina. Based on the colour pattern it is commonly called as brown banded cockroaches. Because of its light body it flies rapidly.
Distribution: India: Karnataka, West Bengal, Maharashtra and Tamilnadu
Remark: Quite uncommon, medium sized species, mostly found in domestic area. Earlier reported from Maharashtra state (Gaikwad et al. 2014).

Family: Blaberidae
Subfamily: Epilamprinae
Genus *Pycnoscelus* Scudder, 1862

4. *Pycnoscelus surinamensis* (Linnaeus, 1758)

1758. *Blatta surinamensis* Linnaeus, Syst. Naturae. 10th Ed. 1: 424.
Material Examined: 1♀, Jashi. Satara Dist, 11. viii. 2018, 2♂Mardi, Satara Dist, 10. viii. 2018.
Diagnosis: Pronotum shiny blackish brown with yellowish margin anteriorly; tegmina dark brwn in colour. Medium size, head with vertex exposed, ocelli large, approximate to the eye. Pronotum laterally rounded,

posterior margin convex. Tegmina and wings extending scarecely upto the apex of the abdomen; subgenital plate with unequally rounded at apex; antrovental margin of front femur with row of slender piliform spinules and terminates in one large spines.

Distribution: India: West Bengal, Tamilnadu, Maharashtra and Karnataka.

Remark: This species generally occurred in decaying vegetation, in day time it hides under dumped vegetation and comes out at night.

Family: Blattidae
Subfamily: Blattinae
Genus *Blatta* Linnaeus, 1758

5. *Blatta orientalis* Linnaeus, 1758

1758. *Blatta orientalis* Linnaeus, Systema natuae, 1 (lOth ed.) Rolmiae: 424.

Material Examined: 1♀Dahiwadi, Satara Dist.,17.viii.2018, 1♂ 1♀ Mardi, Satara Dist , 13.viii.2015.

Diagnosis: The oriental cockroach is a large species of cockroach. It is dark brown to black in colour and has a glossy body. Male head with vertex exposed. Tegmina and wings reduced, covering only about two third of abdominal terga. Anteroventral margin of front. Subgenital plate obtusely rounded at apex.In female cockroach anteroventral margin of front femur with strongly spined. Tegmina short. Hind wings absent. Supra anal plate with mediolongitudinal ridge, posterior margin angulate emerginate. Subgenital plate triangular, lateral margin a little concave. It has a wider body than the male.

Distribution: This species widely distributed throughout the India.

Remark: This species is common in oriental region, generally occurred in domestic area and very less found in natural vegetation, in day time it hides under dumped vegetation and comes out at night.

Genus *Neostylopyga* Shelford, 1911

6. *Neostylopyaga rhombifolia* (Stål, 1813)

1813. *Blatta rhombifolia* Stoll, Repreents. Exact. Coloree d'apres nature d. Specters etc., 2: 5

Material Examined: 2♀Dahiwadi, Satara Dist.,17.viii.2018, 1♀Dahiwadi, Satara Dist.,10.ix.2018,

Diagnosis: Male - Size medim. Vertex little exposed. Pronotum with anterior margin a little convex or entire, posterior margin straight, lateral margin rounded, and maximum width just at the posterior margin. Tegmina reduced. Wings absent. Postterolateral tergite a little indented. Supra anal plate with deeply notched medially. Subgenital plate compressed with obtuse apex. Style thin and slender. In female, anterior margin of pronotum a little concave medially. Posterior margin with maximum width. Tegmina with posterior margin obtusely rounded. Supra anal plate slightly depressed medially.

Distribution: India: Karnataka, Meghalaya, Andaman and Nicobar Islands, Andhra Pradesh, Bihar, Madhya Pradesh, Orissa, Utter Pradesh and West Bengal.

Remark: This species is easily identified by tegminal rudiments with variegated markings. Generally found hided under barks and other suitable part at certain height, and comes out at night.

Genus *Periplaneta* Burmeister, 1838

7. *Periplaneta americana* (Linnaeus, 1758)

1758. *Blatta americana* Linnaeus, Syst. Naturae, (lOth Ed.). 1: 424.

Material Examined: 3♀, Dahiwadi Satara Dist, 18.viii.2018; 2♂ 2♀, Jashi,Satara Dist, 19.viii.2018.

Distribution: All over India.

Remark: This is cosmopolitan species and one of the most important domestic cockroach pest and thrives in tropical and subtropical climates all over the world.

In all over study 7 species observed in which the heavy infestation observed by *Blattella germanica* in domestic as well as in agriculture lands followed by *Periplaneta Americana*. *Blattella humbertiana*, in agricultural lands and *Supella* (*Supella*) *longipalpa* in domestic region found in satisfactory number. *Pycnoscelus surinamensis, Blatta orientalis, Neostylopyaga rhombifolia* found in very less number. Along with these 7 species 2 unidentified species also observed. The record of 7 identified and 2 unidentified species from the drought prone region of Satara district proven that the dry region of Satara district also have a satisfactory diversity. Earlier from the region Blattodea diversity have been never studied therefore this study provides a baseline data for further research.

II. ACKNOWLEDGMENTS:

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QUALITY OF BORE WELL WATER IN ISLAMPUR AREA OF SANGLI DISTRICT (MS), INDIA

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ABSTRACT: *Quality of bore well water in Islampur area of Sangli district, Maharashtra during winter revealed clear water with no odour, temperature (^{0}C) 24, humidity (%) 56, turbidity (N.T.U.) 0.77, total suspended solids (mg/L) 8, total dissolved solids (mg/L) 87, total solids (mg/L) 95, pH 7.7, electrical conductivity (µS /cm) 250, total alkalinity (mg/L) 68, total hardness (mg/L) 58, calcium (mg/L) 12.00, magnesium (mg/L) 8.00, calcium magnesium ratio 1.5, sodium (meq/L) 0.24, sodium (%) 19.23, sodium absorption ratio 37.97, potassium (meq/L) 0.06, chlorides (mg/L) 28.0, carbonates (meq/L) 2.50, bicarbonates (meq/L) 6.00 and residual sodium carbonate 7.24.*

Keywords: *Quality of bore well water, Islampur, Sangli district (M.S.).*

I. INTRODUCTION

World's global water resources accounts for 97.5% out of which 2.5% fresh water resources in which India fresh water resources accounts for 7 to 17% which is inclusive of both surface 64% and ground water resources 36% out of which domestic purpose 5%, industries 6% and agricultural 89% (Chopra and Anwar, 1999). Water is prerequisite for life of human, plants and animals. More than 1 billion people already lack access to safe drinking water (Gleick, 1999). Today water quality model development is taking a new shape especially in the field of river basin management, river dynamics and river water quality. The innate reason is the chain of reactions occurring in the watershed due to increased human influence, leading to changes in the watershed characteristics (Karagul *et al*., 2005).Water is the important source in our earth and plays a vital role in the daily life of every living being. Water which comes as a precipitate into the surface exists in various forms and very few amount of water is being utilized by the living beings (Rao and Kalpana, 2015).

A number of researchers have been worked on various aspects of fresh water (Bhart *et al*. 1999; Gleick, 1999; Adak and Purohit, 2000; Gupta, 2000; Arvind Kumar, 2002; Khabade *et al*., 2002; Harrison *et al*., 2008; Prakash *et al*., 2009; Rajkumar and Nagan, 2010; Gupta *et al*., 2013; Rao and Kalpana, 2015; Sidiqua *et al*., 2016; Mahajan and Pokale, 2017; Shenoy *et al*., 2017; Devojee *et al*. 2018 and Saini and Dube, 2018). The present study was undertaken during winter to attempt some properties of bore-well water which may be significant in drinking purpose water.

II. MATERIALS AND METHODS

Water sample was collected from bore well in Islampur area of Sangli district during winter. Sangli district is surrounded by the district of Kolhapur and Belgum district in the South and Satara and Solapur in the north, Vijapur district in the east and Ratnagiri district in the West. Sangli is located in the western part of Maharashtra between 16 $^o{}_4$ to 17 $^0{}_1$ North Latitudes and 37 $^o{}_{43}$ to 75 $^0{}_{00}$ Eastern Longitude.

Water sample was collected by glass water sampler of one liter capacity (Gupta, 2000; Kumar and Ravindranath, 1998). Water sample was kept in an icebox and stored in the refrigerated. Results were confirmed from government approved Sushilanand Agro Polyclinic Laboratory, Islampur Dist. Sangli. Temperature, pH and odour were measured on the spot. 50 ml of water sample was taken in a 100 ml of clean glass beaker. pH was recorded by pocket pH meter on sampling site. The analysis of all the parameters attempted in the laboratory (Kumar and Ravindranath, 1998; Gupta, 2000 and Kumar and Arvind Kumar, 2002).

Standard turbidity suspension of 100 NTU was prepared from 400 NTU solution. Total dissolved solids of water sample was filtered through a Whatman filter paper No. 4 and sample evaporated at 103 ^{0}C to 105 ^{0}C for 24 hours in an oven, weighed and recorded the reading of beaker with residue. Relative humidity (%) was measured by thermohygrometer. Electrical conductivity of water was measured by standard potassium chloride (0.1 M KCl) in distilled water. In total alkalinity reagents like standard sulfuric acid (H_2SO_4), 0.02N, sodium hydroxide solution (0.02N), phenolphthalein indicator and methyl orange

indicator were used. The titration of 0.02 N sulfuric acid (H_2SO_4) was used until the colour of the solution changed from yellow to orange red. For total hardness buffer solution, inhibitor, eriochrome black T indicator, murexide indicator (ammonium purpurate), sodium hydroxide (2N) and 0.01 M standard EDTA were used and titrated against standard EDTA (0.01 M) till the wine red colour changes to blue. Calcium estimation required titration of EDTA till the pink colour just changed to purple. Magnesium was determined from the values of total hardness and calcium hardness. In the study of total hardness a pinch of the Eriochrome black T indicator was added. Further titration carried out with standard EDTA (0.01 M) till the wine red colour changes to blue. In the determination of calcium hardnessa pinch of murexide indicator was added and immediately titrated with EDTA till the pink colour just changed to purple. Magnesium hardness is the difference between the total hardness and the calcium hardness. Magnesium hardness as $CaCo_3$ = total hardness as $CaCO_3$ - calcium hardness as $CaCo_3$. Magnesium was determined from the values of total hardness and calcium hardness of water. The reagents used in the determination of chlorides included potassium chromate indicator,0.0282 N silver nitrate solution and 0.0282 N sodium chloride. Water sample was titrated against 0.0282 N silver nitrate solution until a brick red precipitate was formed. For better accuracy 50 ml of distilled water was titrated against 0.0282 N silver nitrate solution and established blank. In determination of sodium a series of standard sodium chloride solutions were prepared. Air pressure of flame photometer was kept at 10 Ibs and blue sharp flame cone was obtained. Fed different standard sodium solutions one by one but maximum at first and record the readings of standards. Then fed water sample and noted the flame photometer reading. Plotted a standard curve between standards (concentrations) of Na (me/L) and flame photometer readings of standard of Na. Obtained concentration of Na in unknown water sample from the standard curve. In the determination of potassium a series of potassium chloride standard solutions were obtained and concentration of K in unknown water sample were obtained from the standard curve. Determination of per cent sodium was carried out according to Kumar and Arvind Kumar (2002). The values of individual constituents were taken in milliequavalent per liter. Equivalent weights of Ca (20), Mg (12), Na (23) and K (39) (Larousse, 1866) were used in the conversion of mg/L into meq/L. Sodium adsorption ratio was calculated according to Kumar and Arvind Kumar (2002).

III. RESULTS AND DISCUSSION

RESULTS

Quality of bore well water in Islampur area of Sangli district, Maharashtra during winter revealed clear water with no odour, temperature (^{0}C) 24, humidity (%) 56, turbidity (N.T.U.) 0.77, total suspended solids (mg/L) 8, total dissolved solids (mg/L) 87, total solids (mg/L) 95, pH 7.7, electrical conductivity (µS /cm) 250, total alkalinity (mg/L) 68, total hardness (mg/L) 58, calcium (mg/L) 12.00, magnesium (mg/L) 8.00, calcium magnesium ratio 1.5, sodium (meq/L) 0.24, sodium (%) 19.23, sodium absorption ratio 37.97, potassium (meq/L) 0.06, chlorides (mg/L) 28.0, carbonates (meq/L) 2.50, bicarbonates (meq/L) 6.00 and residual sodium carbonate 7.24.

DISCUSSION

The quality of bore well water varies from place to place; even at the same location, from season to season. It also depends upon both the surface and subsurface characteristics. Physico-chemical features of Cauvery river water have been studied by Prakash *et al.* (2009). Gupta *et al.* (2013) noted physico-chemical properties of Yamuna River with pH 7.3 to 7.7, electrical conductivity range of 990 µ mhos/cm to 1285µ mhos/cm, total hardness values ranged from 252 mg/l to 304 mg/l,. sodium varied between 404.9 mg/l to 524.0 mg/l, potassium concentration ranged between 18.1 mg/l to 23.8 mg/l. Shenoy *et al.* (2017). Quality of bore well water in Udupi Muncipal area revealed total dissolved solids (mg/L) 183.37, pH 6.3, electrical conductivity (µS /cm) 374, total alkalinity (mg/L) 77.82, total hardness (mg/L) 77.24, calcium (mg/L) 40.3, magnesium (mg/L) 8.19, sodium (mg/L) 39, potassium (mg/L) 11.32 and chlorides (mg/L) 14.9. Saini and Dube (2018) have been noted B.O.D. values ranged between 5.00to 13.00 mg/l. The maximum value of 13.00 mg/l is recorded in the month of August 2011.

In the present work clear water indicates absence of suspended matter. Temperature affects the chemical and biological reactions in water. A rise in temperature of water accelerates chemical reactions, reduces solubility of gases, amplifies taste, odour and elevate metabolic activity of organisms. Turbidity 0.77 (N.T.U.) indicates turbidity within highest desirable limit. The turbidity in water is mainly caused by sand, silt, clay, microorganism or organic material dissolver in it. Turbid water absorbs more sunlight and hence the temperature of water increases. Total dissolved solids 87 mg/L indicates dissolved solids within

permissible limit. In the present study pH 7.7 indicates alkaline nature of water. Beyond pH 8.2 water will affect the mucous membrane and corrosion in the boilers and other metallic pipes, hence their determination is important for drinking purpose water. In unpolluted water pH is controlled by the balance between dissolved carbon dioxide and the carbonate and bicarbonate ions. Electrical conductivity 250 µS/cm values indicating the presence of low amount of dissolved inorganic substances in ionized form. Conductivity is an indirect measure of the salt concentration (salinity). Total alkalinity is a measure of the quantity of ions that react to neutralize hydrogen ions. Total alkalinity is the sum of hydroxides, carbonates and bicarbonates. Total alkalinity is useful in assessing the water used for irrigation and potability of drinking water. Alkalinity increases as the amount of dissolved carbonates and bicarbonates increase. Total Hardness values 58 mg/L found within the permissible limit of WHO. Waters become hard primarily due to excessive presence of bicarbonate, chloride and dissolved sulphate. This water sample is suitable for drinking purpose and for cooking due to low boiling point. Low hardness of water may be due to presence of low alkaline earth metal cations, mainly calcium and magnesium leaching of rocks in the catchments. Calcium is an important component in the exoskeleton of arthropods and shells of mollusks while magnesium is vital component of chlorophyll. High concentration of magnesium imparts an unpleasant taste to the potable water and adversely affects on domestic use. In the present work alkalinity which was found greater than hardness suggests presence of basic salts of sodium and potassium, in addition to calcium and magnesium. Calcium 12 mg/L and magnesium 8 mg/L suggest Ca:Mg ratio is more than one preferably and within desirable limit. Sodium (meq/L) 0.24, sodium (%) 19.23 and sodium absorption ratio 37.97 suggest water is also suitable for irrigation. The presence of sodium is also an important parameter, the excess quantity of which can deteriorate the soils. High value of sodium may also damage the sensitive crops because of sodium toxicity. Sodium absorption ratio greater than13 are usually considered to be sodic. Chlorides 28 mg/L suggests suitability of water according to Indian Standards Institute. Carbonates (meq/L) 2.50, bicarbonates (meq/L) 60 and residual sodium carbonate 7.24 indicate carbonates, bicarbonates and residual sodium carbonate are within permissible limit.

It is concluded from the present study that physical and chemical characteristics of the bore well water shows the value within permissible limit of standards of World Health Organization (WHO), Bureau of India Standards and Ministry of Works and Housing Gupta (2000) and Indian Standards Institute (Kumar and Ravindranath, 1998). Water can be used for drinking purpose only after proper treatment and directly for irrigation. Our results are in good agreement with the findings of above authors.

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STUDY OF ZOOPLANKTON DIVERSITY OF WATER BODIES OF KARVE AND KADEGAON, DIST. SANGLI (M.S) INDIA

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ABSTRACT: *The present investigation deals with the study of zooplankton diversity of Sonhira lake, Kadegaon and Karve lake, Karve near Vita city of Sangli district, Maharashtra. The study was carried out for the period of one year i.e. June 2017 to June 2018. In laboratory all preserved specimens were taxonomically identified. Results of zooplankton reveals that total number of 31 and 21 species of Zooplankton belonging to 5 and 4 different groups were recorded from Sonhira and Karve lake respectively. Zooplanktons diversity was found rich Sonhira lake as compare to Karve lake.*

Keywords: *Diversity, Zooplankton, Karve, Kadegaon*

I. INTRODUCTION

Zooplanktons are microscopic, floating and drifting animals with limited power of locomotion (Ahlstrom,1936). The zooplankton abundance and their diversity are used to determine the health of aquatic ecosystem (MBO, 2007). Zooplankton diversity is one of the most important ecological parameters in water quality assessment. Earlier studies reported, various indices like richness, diversity and evenness index can be calculated with the data on taxonomy of different zooplankton (Sakhare, 2007). The study of zooplankton diversity indices contributes to an understanding of the environmental status of a water body. Numerous studies enriching the existing data regarding the changes of zooplankton, composition and abundance in response to tropic gradients and zooplankton biomass dynamics (Santos et al.,1994) .Additionally, beyond nutrient availability, zooplankton can be regulated by top-down effects (Santos et al., 1994). Zooplankton acts as bioindicator of changes in water quality status, because it is quickly responded to changes in environmental conditions (Gannon and Stemberger, 1978). Zooplankton comprising of rotifers, cladocerans, copepods and ostracods are considered to be most important in terms of population density, biomass production, grazing and nutrient regeneration in any aquatic ecosystem. Their diversity and density is mainly controlled by availability of food as favorable water quality (Chandrasekhar and Kodarkar, 1997).

Taking account of literature and carried work, we have decided to assess planktonic diversity from sonhira lake of kadegoan and karve lake of vita in terms of taxonomic richness and density which would help in understanding diversity indices of biological community.

So the present study was carried out to understand the diversity of zooplankton from Sonhira lake of Kadegaon & Karve lake , Sangli District, Maharashtra. (India).

II. MATERIAL AND METHODS

Study Area-

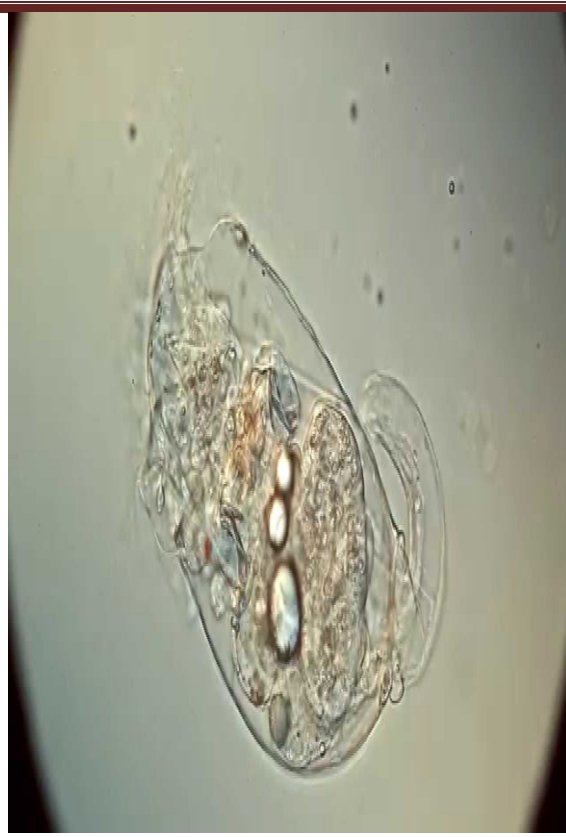
Sonhira lake is located in Sonkire village of Kadegaon, Sangli district, Talukha Kadegaon Maharashtra, India. & Karve lakes is located in karve village of vita district sangli, talukha Khanapur. The climatic conditions of the study area was hot in summer and cool in winter and temperature range a minimum 24ºC and a maximum of 42ºC. Vita is nearest town to Sonkire which is approximately 40km away. The study area gets most of its rainfall from June to September during the monsoon. The selected water bodies are of perennial type.

Collection of sample –

Sample collection was done by using various types of zooplankton nets, sample collection carried out from June 2017 to July 2018. By following standard method all samples were persevered in 4% formalin. All samples then brought to the laboratory and taxonomically identified under light microscope. Morphological identification was carried out by following standard keys.

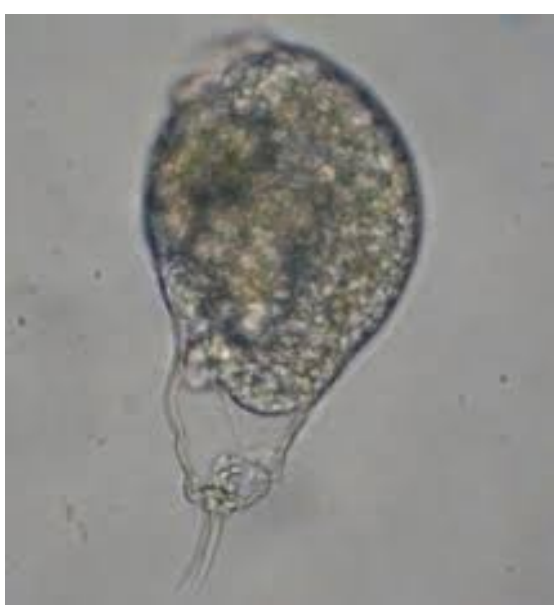
Karve lake in vita

Sonhira lake in Kadegaon

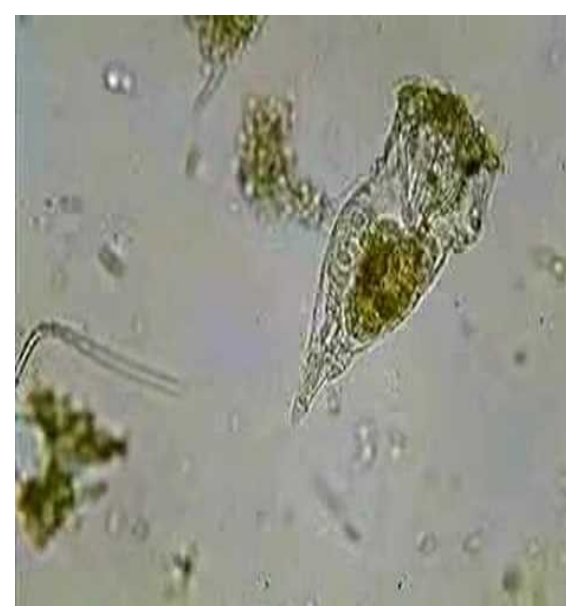


1.Brachionuscalyciflorus

2.Brachionus angularis

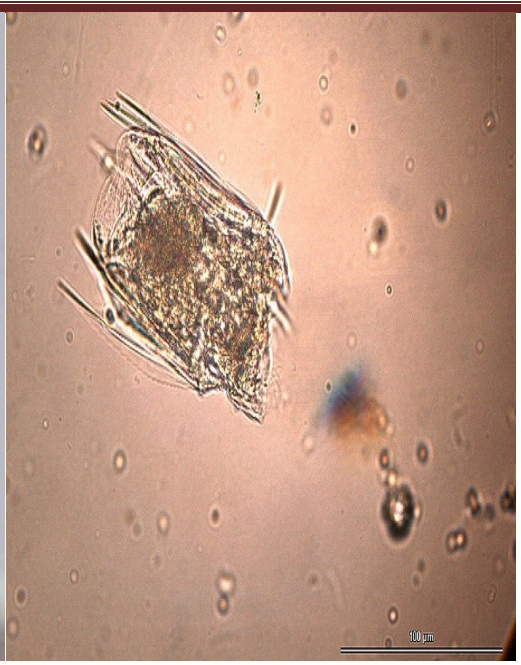


3.Gastropus minor



4.Synchaeta sp.

5.Polyarthra vulgaris



6.Monostyella sp.

Name of species	Site in Kadegoan	Site in Karve
Rotifera		
Brachionus calyciflorus	+	+
Brachionus angularis	+	+
Keratella cochlearis	+	+
Lecanelunaris	+	+
Gastropus minor	+	+
Ascomorphaovalis	+	+
Synchaetasp.	+	+
Polyarthra vulgaris	+	+
Philodina citrine	+	+
Keratell acochlearis	+	+
Brachionus calyciflorus	+	+
Filinia longiseta	+	+
Lecanidae Lecanelunaris	+	-
Polyarthra vulgaris	+	-
Monostyella sp	-	+
Branchionus Caudatus	-	+
Cladocera		
Daphania pulex	+	+
Daphania carinata	+	+
Monia brachiata	+	+
Bosmina Sp	+	+
Alona pulchella	+	+
Daphnia carinata	+	+
Monia brachiata	+	+
Moina micrura	+	+
Diaphanosoma excisum	-	+

Daphnia longirimis	-	+
Copepoda		
Moinamacrocopa, Straus	+	+
Daphnia gessneri Herbst	+	+
Kurzia longirostris Daday	+	+
Moina sp	+	+
Cyclopoidcopepodite	+	+
Diaptomuspallidus	+	+
Cyclops sp.	-	+
Mesocyclops sp	-	+
Protozoa	+	+
Paramecium cadatum	+	+
Vorticella companula		
Ostracoda	-	+
Cypris protubera Muller	-	
Cyprinus nudus Brady		

III. RESULT AND DISSCUTION

In the present study of Kadegoan sonhira lake, total 31 species belonging to 4 different groups were found under different climatic conditions. Out of 31 species,14 species of rotifera, 10 species of Cladocera,8 species of copepod and 2 species of protozoa were recorded during the study period. Zooplankton diversity was found rich during summer as compare to rainy and winter season.

Karve lake,total species to 5 different groups were found abundant under different climatic condition. Out of 21 species 14 species of rotifera, 9 species of Cladocera 8 species of copepod 2 species of protozoa , 2 species of Ostracoda.

Mahor R.K.(2011), Krishnamoorthi, A. and Selvakumar S. (2012) were studied the protozoan diversity and abundance. They were found maximum density of Protozoa in summer and winter months and minimum in monsoon months. The rotifera were found in maximum numbers during the winter and summer season but minimum number were found during the monsoon. According to Adoni,(1985),the density of rotifera as well as their diversity increases due to increase in eutrophication. Gannon and Stemberger, (1978), Singh and et.al., (2002), studied on density and biomass of cladocerans and their finding was density of cladocerans correlates with food supply. According to Chauhan, R (1993), the average density of Copepods were reported maximum during summer season and minimum during monsoon season. Similar findings were observed by Thirupathaiah, et. al., (2012).

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STUDY OF AVIFAUNA IN AND AROUND VITA CITY, DIST. SANGLI, (M.S.) INDIA

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ABSTRACT: *Present paper deals with thestudy of avifauna in and around Vita City, Dist. Sangli (M.S.).The observations of the birds are carried out according to the standard method prescribed by Ali (2002). The preparation of check list of birds is carried out according toMankadan and Pittie (2002).Total 92 species of bird representing 46 families are observed.Out of 92 species 80 are resident, 4 are local migrant, 7 are winter migrant and 1 is foreign migrant. Out of total birds species sighted in present study three are near threatened. The study area also includes two endemic bird species. The results are discussed with available literature.*

Keywords: *Avifauna, Check list, Vita City.*

I. INTRODUCTION

Birds (Aves) are feathered bipeds, a group of endothermic vertebrates having toothless beaked jaws, the laying of hard shelled eggs, a high metabolic rate, a four-chambered heart and a strong yet light weight skeleton. Birds live worldwide and range in size from the 5 cm (2 inch) to the 2.75 m (9 foot). Birds are only amniotes whose body is highly specialized for aerial mode of life. The total number of bird species on earth known is about 8600. While in Indiathe total number of bird species is 1263 (Kotpal,1989).

Many bird species migrate to take advantage of global differences of seasonal temperatures, therefore optimizing availability of food sources and breeding habitat. Before migration, birds substantially increase body fats and reserves and reduce the size of some of their organs(Ali, 2002).

Various aspects of avifauna were studied by different workers like roosting behaviour of crow at Shivaji Nagar, Barshi (Kamble and Fartade, 2012), avifauna of summer hill, Simla, Himachal Pradesh (Kulkarni and Goswami, 1912), a report of avifaunal diversity around Buldhana town (Kakde, 2012) etc.,however the information on avifauna of Vita city is rather scanty. There exists lacuna in the field of study of avifauna in and around Vita city. Therefore the present study provides information on avifauna of Vita city. It also helps to prepare checklist of avifauna of Vita city.

II. MATERIALS AND METHODS

2.1 Materials: Study area:

Vita city is one of the big city in a Sangli district in Maharashtra state. It is nicknamed as "City of Gold" It is situated on two state highways *viz*., Guhagar- Bijapur and Ahamednagar- Sangli. Vita city is situated on 17.2711 latitude and 74.5378 longitude. It belongs to Desh or Paschim Maharashtra region. Total land area covered by Vita is 55.3 $km^{2.}$ Total population of Vita city is 48,289. The average temperature of Vita city is ranges from 14 to 40^{o}C. Climate of the Vita is dry and average rainfall is 80 -120 mm.

In spite of water scarcity, people in Vita have displayed entrepreneurship in jewellary, agriculture, textile, poultry farming, hoteling. Some waterbodies are present around Vita city *viz.,*Vivekanandnagar lake, Alasand lake, Bhambarde lake. The crops around Vita are mainly cash crops, wine yards and sugarcane. The crops also includecultivation of jawar, bajara, weat, soyabean, etc.

2.2 Methods:

Opportunistic visits and survey were carried out in above mentioned selected areas on multiple occasions during the November 2016 to October 2017. Observations were carried out both in the morning and evening between 06:00 – 10:00 hrs. and 16:00 – 18:00 hrs., when the birds were most active. The bird species were recorded by direct sighting and calls. The birds were directly observed by 10 X 50X olympus binocular and identified using field guide by Ali (2002). The checklist is prepared following Mankadan and Pittie (2002). Survey was made along the edges of water bodies, hills, forest paths, in agriculture fields and near the human habitations.

Depending on movement and seasonality of occurrence, the birds are classified as resident (species found in the study area throughout the year), local migrant (species found in the study area irregularly, but is resident of India), winter migrant (species found in the study area only during winter

season). Depending on the frequency of sightings during the field visits, birds are classified as common (species observed repeatedly in suitable habitat), uncommon (species occurs on regular basis but not frequently in suitable habitat). Status of threatened category was adopted from Bird Life International (2016).

III. RESULTS AND DISCUSSION

This study reports the occurrence of 92 species of birds representing 46 families. Out of 92 species 80 are resident,04 are local migrant, 07 are winter migrant and 01 foreign migrant species. Out of total birds species sighted in present study 03 are near threatened namely, Black headed Ibis, *Threskiornismelanocephalus*, Painted Stork, *Mycterialeucocephala* and River Tern, *Sternaaurantia*. The study area also includes 02 endemic bird species namely, White-throated Faintail Flycatcher, *Rhipiduraalbicolis* and Indian Yellow Tit, *Parusaplonotus*.

Table 1: Checklist of Avifauna of Vita city, Dist. Sangli (M.S.)
Status: R- Resident, LM- Local Migrant, WM- Winter Migrant, LC- Least Concern, NT- Near Threatened.

Sr. No.	Family and Common Name	Scientific Name	Migratory Status	IUCN Status	Occurrence
	Family, Podicipedidae				
1	Little Grebe	*Tachybaptus ruficollis*	R	LC	Common
	Family, Phalacrocoracidae				
2	Little Carmorant	*Phalacrocorax niger*	R	LC	Common
	Family, Ardeidae				
3	Little Egret	*Egretta garzetta*	R	LC	Common
4	Grey Heron	*Ardea cinerea*	R	LC	Common
5	Purple Heron	*Ardea purpurea*	R	LC	Uncommon
6	Cattle Egret	*Bubulcus ibis*	R	LC	Common
7	Indian Pond Heron	*Ardeola grayii*	R	LC	Common
8	Great Egret	*Ardea alba*	LM	LC	Uncommon
	Family, Ciconiidae				
9	Painted Stork	*Myctaria leucocephala*	LM	NT	Uncommon
10	Woolly-Necked Stork	*Ciconia episcopus*	R	LC	Common
	Family, Threskiornithidae				
11	Black Headed Ibis	*Threskiornis melanocephalus*	WM	NT	Uncommon
12	Indian Black Ibis	*Pseudibis papillosa*	R	LC	Common
13	Eurasian Spoonbill	*Platalea leucorodia*	WM	LC	Common
	Family, Anatidae				
14	Indian Spot-billed Duck	*Anas poecilorhyncha*	R	LC	Common
	Family, Accipitridae				
15	Black-Winged Kite	*Elanus caeruleus*	R	LC	Common
16	Black Kite	*Milvus migrans*	R	LC	Common
17	Brahminy Kite	*Haliastur indus*	R	LC	Common
18	Crested Serpent Eagle	*Spilarnis cheela*	R	LC	Common
19	Shikra	*Accipiter badius*	R	LC	Uncommon
	Family, Phasianidae				
20	Common Quail	*Coturnix coturnix*	R	LC	Common
21	Indian Peafowl	*Pavo cristatus*	R	LC	Common
	Family, Rallidae				
22	White-Breasted Waterhen	*Amaurornis phoenicurus*	R	LC	Common

23	Eurasian Coot	*Fulica atra*	R	LC	Common
24	Common Moorhen	*Gallinula chloropus*	LM	LC	Uncommon
	Family, Charadriidae				
25	Yellow-Wattled Lapwing	*Vanellus malabaricus*	WM	LC	Uncommon
26	Red-Wattled lapwing	*Vanellus indicus*	R	LC	Common
	Family,Laridae				
27	River Tern	*Sterna aurentia*	R	NT	Common
	Family, Scolopacidae				
28	Wood sandpiper	*Tringa glareola*	WM	LC	Uncommon
29	Common Sandpiper	*Actitis hypoleucos*	R	LC	Common
	Family, Recurvirostridae				
30	Black-Winged Stilt	*Himantopus himantopus*	R	LC	Common
	Family, Columbidae				
31	Rock Pigeon	*Columba livia*	R	LC	Common
32	Laughing Dove	*Streptopelia senegalensis*	R	LC	Common
33	Spotted Dove	*Streptopelia chinensis*	R	LC	Common
34	Eurasian collard Dove	*Streptopelia decaocto*	R	LC	Common
	Family, Psittacidae				
35	Rose-Ringed Parakeet	*Psittacula krameri*	R	LC	Common
36	Plum-Headed Parakeet	*Psittacula cyanocephala*	R	LC	Common
	Family, Cuculidae				
37	Asian Koel	*Eudynamys scolopacea*	R	LC	Common
38	Greater Coucal	*Centropus sinensis*	R	LC	Common
	Family, Strigidae				
39	Spotted Owlet	*Athene brama*	R	LC	Common
	Family, Tytonidae				
40	Barn Owl	*Tyto alba*	R	LC	Common
	Family, Apodidae				
41	Little Swift	*Apus affinis*	R	LC	Common
42	House Swift	*Apus nipalensis*	R	LC	Common
	Family, Alcedinidae				
43	Common Kingfisher	*Alcido atthis*	R	LC	Common
44	Pied Kingfisher	*Ceryle rudis*	R	LC	Common
45	White-Throated Kingfisher	*Halcyon smyrnensis*	R	LC	Common
	Family, Meropidae				
46	Small Green Bee-Eater	*Merops orientalis*	R	LC	Common
	Family, Coraciidae				
47	Indian Roller	*Coracias benghalensis*	R	LC	Common
	Family, Upupidae				
48	Common Hoopoe	*Upupa epops*	R	LC	Common
	Family, Bucerotidae				
49	Indian Grey Hornbill	*Ocyceros birostris*	R	LC	Common
	Family, Capitonidae				
50	Coppersmith Barbet	*Megalaima haemacephala*	R	LC	Common
	Family, Alaudidae				

51	Ashy-Crowned Sparrow Lark	*Eremopterix griseus*	R	LC	Common
52	Common Crested Lark	*Galerida cristata*	R	LC	Common
	Family, Hirundinidae				
53	Wire-Tailed Swallow	*Hirundo smithii*	R	LC	Common
54	Red-rumped Swallow	*Hirundo daurica*	R	LC	Common
	Family, Motacillidae				
55	Yellow Wagtail	*Motacilla flava*	WM	LC	Common
56	White Wagtail	*Motacilla alba*	WM	LC	Common
57	White-browed Wagtail	*Motacilla maderaspatensis*	R	LC	Common
	Family, Campephagidae				
58	Small Minivet	*Pericrocotus cinnamomeus*	R	LC	Common
	Family, Pycnonotidae				
59	Red-Vented Bulbul	*Pycnonotus jocosus*	R	LC	Common
	Family, Irenidae				
60	Common Iora	*Aegithina tiphia*	R	LC	Common
	Family, Laniidae				
61	Bay-Backed Shrike	*Lanius vittatus*	R	LC	Common
	Family, Turdinae				
62	Oriental Magpie-Robin	*Copsychus saularis*	R	LC	Common
63	Pied Bushchat	*Saxicola caprata*	R	LC	Common
64	Indian Black Robin	*Saxicoloides fulicata*	R	LC	Common
	Family,Leiothrichidae				
65	Yellow-eyed Babbler	*Chrysomma sinense*	R	LC	Common
66	Large Grey Babbler	*Tudoides malcolmi*	R	LC	Common
	Family, Cisticolidae				
67	Grey-Breasted Prinia	*Prinia hodgsonii*	R	LC	Common
68	Plain Prinia	*Prinia inornata*	R	LC	Common
69	Ashy Prinia	*Prinia socialis*	R	LC	Common
70	Common Tailorbird	*Orthotomus sutorius*	R	LC	Common
	Family, Muscicapidae				
71	Tickell's Blue Flycatcher	*Cyornis tickelliae*	R	LC	Common
72	Red throated flycatcher	*Ficedula parva*	WM	LC	Occasional
	Family, Rhipiduridae				
73	White-Throated Fantail	*Rhipidura albicollis*	R	LC	Common
	Family, Paridae				
74	Great Tit	*Parus major*	R	LC	Common
75	Indian Yellow Tit	*Parus aplonotus*	R	LC	Common
	Family ,Nectariniidae				
76	Purple-Rumped Sunbird	*Nectarinia zeylonica*	R	LC	Common
77	Purple Sunbird	*Nectarinia asiatica*	R	LC	Common
	Family, Zosteropidae				
78	Oriental White Eye	*Zosterops palpebrosus*	R	LC	Common
	Family, Estrildidae				
79	Red Avadavat	*Amandava amandava*	LM	LC	Uncommon
80	Scaly Breasted Munia	*Lonchura punctulata*	R	LC	Common
81	Tricolored Munia	*Lonchura malacca*	R	LC	Common
	Family, Passeridae				

82	House Sparrow	*Passer domesticus*	R	LC	Common
	Family, Ploceidae				
83	Baya Weaver	*Ploceus phillipinus*	R	LC	Common
84	Streaked Weaver bird	*Ploceus manyar*	R	LC	Common
	Family, Sturnidae				
85	Brahminy Starling	*Sturnus pagodarum*	R	LC	Common
86	Common Myna	*Acridotheres tristis*	R	LC	Common
87	Jungle Myna	*Acridotheres fuscus*	R	LC	Common
88	Rosy Starling	*Pastur roseus*	M	LC	Uncommon
	Family, Dicruridae				
89	Black Drongo	*Dicrurus macrocerus*	R	LC	Common
90	White-Bellied Drongo	*Dicrurus caerulescens*	R	LC	Common
	Family, Corvidae				
91	House Crow	*Corvus splendens*	R	LC	Common
92	Jungle Crow	*Corvus macrorhynchos*	R	LC	Common

Status: R- Resident, LM- Local Migrant, WM- Winter Migrant, LC- Least Concern, NT- Near Threatened.

IV. CONCLUSION

Based on our observations we have concluded that, the study area having rich vegetation and number of water bodies which provides shelter and roosting sites to the different birds. It also provides good habitat and breeding ground which attract different local and migratory birds. Considering above facts there is a need to aware the local peoples about richness of these places and takes the steps towards the conservationof these favorable resources of avifauna.

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DIVERSITY AND DISTRIBUTION OF AGROBIONT SPIDERS (ARACHNIDA: ARANEAE) FROM DIFFERENT AGRO-ECOSYSTEMS OF ANJANI VILLAGE, M.S. (INDIA)

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ABSTRACT: *The present paper deals with a study of diversity and distribution of agrobiont spiders from different agro-ecosystem viz., Banana,Sugar- apple, Sorghum and Vineyard of Anjani village, Dist. Sangli (M.S.), India.The study was conducted for 11 months from January 2018 to November 2018. A Total 41 species of spiders belonging 25 genera and 10 families were recorded during the study period. The family Araneidae (31.70%)with 13 species was the most dominant followed bySalticidae (14.63%), Thomisidae (14.63%), Lycosidae (12.19%), Oxyopidae (07.31%), Sparassidae (07.31%), Tetragnathidae (4.87%), Hersiliidae (2.43 %),Pissauridae (2.43 %) and Uloboridae (2.43 %). The diversity index has shown that the species diversity is maximum intheSugar- appleagro-ecosystem (Shannon Index-3.434) followed by Banana and Sorghum (Shannon Index- 3.178). The less spider diversity was found in a vineyard (Shannon Index-2.445). The species richness was being highest in Sugar- apple agro-ecosystem(Margalef's Index – 8.736) whereasit was found less at the vineyard agro-ecosystem (Margalef's Index – 4.427). The results are analyzed with presently available literature.*

Keywords: *Agrobiont, Spider,Anjani Village,Agro-ecosystem,Vineyard.*

1. INTRODUCTION

The cumulative studies made on agrobiont spider diversity in four different agro – ecosystem habitats of Anjani Village of Sangli district. Spiders are one of the most ubiquitous, diverse and seventh largest groups of animal. At present total, 47439 species of spiders are recorded in the world (Platinick: World spider catalog, 2018) and total 1698 species are recorded in India (Keswani *et al.,* 2012). A total of 90 species belonging to 55 genera and 19 families are recorded from Zolambi Region of Chandoli National Park which came under the Sangli District (More, 2015).Spiders are generalist predators they act as biological control agents within agro - ecosystems (Riechert and Bishop, 1990; Young Kajak, 1997).

Spiders play an important role in controlling insect pest in the agro- ecosystem. Spiders are considered to be the friends of farmers as they play important role in crop pest management by consuming a large number of pest without damaging the crop (Rajeshwaram, 2005). As far as the predatory role of spiders is concerned they divided into two groups "Web – weavers" (Construct the web and hunts the preys caught in the web) and "Active hunter" (Hunts the prey by searching). Among the families recorded in the present study, family Araneidae, Tetragnathidae, and Uloboridae are the Orb web weavers and rests of all are belonging to the active hunters. The aim of present study is to find out the current status of spider diversity from four different agro- ecosystem habitats, for the purpose of understanding their predatory role and conservation of spiders species present in agro-ecosystem.

II. MATERIALS AND METHODS

2.1 Materials: Study area:

The present study was carried out in the limited area of four different agro-ecosystem habitats *viz.,*Vineyard, Banana, Sorghum and Sugar- apple of Anjani village. It is the 24th most populous village, locatedin Tasgaon tehsil, district Sangli in the state Maharashtra (India). The total geographical area of Anjani village is 12 km^2 and it is the 24th biggest village by area in the sub-district.Anjani village is situated on 17.0781954 N and 74.776058 E. The average temperature of Anjani ranges from 20^{0}C to 40^{o}C. A climate of the Anjani is dry and average rainfall is 80 -120 mm. The main cash crop like a vineyard, sugarcane, banana, and some seasonal crops are cultivated.

For the present study total 12 acres area of four different agro-ecosystem habitats (each 03 acres) *viz.,*Banana,Sugar- apple, Sorghum and Vineyard were selected.

2.2 Data Collection:

The survey was conducted from January 2018 to November 2018 for different agriculture habitats. Spiders were collected using standard sampling techniques such as sweep netting, hand picking, and umbrella collection. All survey was collected by a weekly visit to study area in the morning hours (07.00 am to 10.00 am) and evening hours (05.00 pm. to 07.00pm.).Species were photographed by using Nikon D5300 camera (lens 18-55mm, with macro extension tube) and identified in their natural habitat. When it was difficult to assess, then specimen were captured for their further identification and after identification, they were released in their natural habitat immediately.

The identification was done by the standard identification key prescribed by Pocock (1900), Tikader (1982), Biswas and Biswas (2003) and Sebastian and Peter (2009) in the book of Spiders of India.

2.3. Statistical Analysis:

1. **Diversity is calculated by using Shannon-Weaver index (H`).**

Formula $H` = - \Sigma Pi \ In \ Pi$

Where,

$Pi = S/N$

S = Numbers of individuals of one species

N = Total number of all individuals

In = Logarithm to base e.

2. **Evenness of the species is calculated by using Pielou's evenness index**.

The evenness index ranges from zero to one, where zero signifying no evenness and one as complete evenness.

Formula $e = H/ \ In \ S$

Where,

H = Shannon- Weaver index.

3. **Species richness is calculated by using Margalef's index**

Formula- Margalef's index = S – 1/ In N

III. RESULTS

A total of 41 species of spiders belonging to 25 genera and 10 families were recorded from four different agricultural habitats *viz.,*Banana,Sugar- apple, Sorghum and Vineyard (Table No. 01 and 02). The family Araneidae (31.70%) was most abundant family represents 06 genera and 13 species followed by Salticidae (14.63%) represents 06 genera and 06 species, Thomisidae (14.63%) represents 02 genera and 06 species, Lycosidae (12.19%) represent 03 genera and 05 species, Oxyopidae (07.31%) represents 01 genera and 03 species, Sparassidae (07.31%) represents 02 genera and 03 species, Tetragnathidae (4.87 %) represents 02 genera and 02 species, Hersiliidae (2.43 %)represents 01 genera and 01 species,,Pisauridae (2.43 %) represents 01 genera and 01 species and Uloboridae (2.43 %) represents 01 genera and 01 species (Table No. 01.,Fig. No.01 and Fig. No.02). TheSugar- apple agriculture habitatwas recorded highest species diversity (Shannon Index-3.434) followed by Banana and Sorghum agricultural habitat (3.178). The less diversity was found in the vineyard (Shannon Index-2.445) (Table No.02 and 03). The species richness was being highest in Sugar- apple agriculture habitat(Margalef's richness Index – 8.736)(Table No.02 and 03) whereasit was found less in the vineyard habitat (Margalef's richness Index – 8.736)(Table No.02 and 03).The species evenness wasfound same to all four agro -ecosystem (Pielou's Evenness Index - 01). These results indicate that the diversity and species richness of spiders were found less in vineyard habitat because of overuse of pesticide and fertilizers.

IV. DISCUSSION

A total of 41 species of spiders belonging to 25 genera and 10 families were recorded from four different agricultural habitats*viz.,* Banana, Sugar- apple, Sorghum and Vineyard (Table No. 01 and 02). The family Araneidae (31.70%) was most abundant family represents 06 genera and 13 species.

Keswani and Vankhede (2014) were recorded 50 species of spiders from 39 genera and 15 families from Banana agro-ecosystem, they also observed the family Araneidae was most abundant family. More (2015) recorded the 90 species of spiders from Zolambi region of Chandoli National Park and reported family Araneidae was most abundant family. Asarkar (2017) studied the diversity of spiders from three agro- ecosystems viz., Banana, cotton, citrus and reported family Araneidae was the most abundant family. Our results are also in good agreement with the findings ofNyffeler (1994), Solanki and Kumar (2015), Ambily and Antony (2016), Wankhade et al.(2012) and Prajapatiet al. (2018).

Table No. 01 Species distribution of agrobiont spiders of different families in four agro-ecosystems

Sr.No.	Family	Genera	Species	Species %
01	Araneidae	06	13	31.70 %
02	Hersiliidae	01	01	2.43 %
03	Lycosidae	03	05	12.19 %
04	Oxyopidae	01	03	07.31 %
05	Pisauridae	01	01	02.43 %
06	Salticidae	06	06	14.63 %
07	Sparassidae	02	03	07.31 %
08	Thomisidae	02	06	14.63 %
09	Tetragnathidae	02	02	04.87 %
10	Uloboridae	01	01	02.43 %
	Total	**25**	**41**	**100 %**

Table No. 02: List of Spiders recorded from four agro-ecosystems

Sr.No.	Spider Species	Agro-ecosystems			
		Banana	Sugar-apple	Sorghum	Vineyard
I.	**Family - Araneidae**				
01	*Araneus mitificus* (Simon, 1886)	×	√	×	×
02	*Argiope aemula* (Walckenaer, 1841)	√	×	√	×

03	*Argiope anasuja* (Thorell, 1887)	√	×	√	×
04	*Cyclosa bifida* (Doleschall, 1959)	√	√	√	√
05	*Cyclosa moonduensis* (Tikader, 1963)	√	√	×	×
06	*Cyclosa insulans* (Costa, 1934)	×	√	×	×
07	*Cyclosa confraga* (Thorell, 1892)	√	√	×	×
08	*Cyrtophora citricola* (Forsskal,1775)	×	√	×	×
09	*Neoscona mukerajei* (Tikader, 1980)	√	√	√	√
10	*Neoscona nautica* (C. L. Koch, 1875)	√	√	√	√
11	*Neoscona bengalensis* (Tikader and Ball,1981)	√	√	×	×
12	*Neoscona theisi* (Walckenaer,1842)	√	√	√	×
13	*Thelacantha brevispila* (Doleschall, 1857)	×	√	×	×
II.	**Family- Hersiliidae**				
14	*Hersilia savignyi* (Lucas, 1836)	×	√	×	√
III.	**Family- Lycosidae**				
15	*Perdosa oriens*(Chamberlil, 1924)	√	√	√	×
16	*Perdosa pseudoannulata* (Bosenberg and Strand, 1906)	√	√	×	×
17	*Lycosa poonaensis* (Tikader and Malhotra, 1980)	√	√	√	×
18	*Hippasa greenalies*(Blackwall, 1967)	√	√	√	√
19	*Hippasa spe.*	√	√	√	√
IV.	**Family- Oxyopidae**				
20	*Oxyopes javanus* (Thorell, 1887)	√	√	√	√
21	*Oxyopes pankaji* (Gajabe and Gajabe, 2001)	√	√	√	×
22	*Oxyopes shweta* (Tikader,1970)	×	√	×	×
V.	**Family- Pissauridae**				
23	*Nilus marginatus* (Simon, 1888)	×	×	√	×
Sr.No.	**Spider Species**	**Agricultural Habitat**			
		Banana	**Sugar-apple**	**Sorghum**	**Vineyard**
VI.	**Family - Salticidae**				
24	*Chrysilla stipesa*	√	×	√	×
25	*Epeus indicus* (Proszynscai, 1992)	×	×	√	×
26	*Myrmarachne spe.*	√	√	√	√
27	*Phintella vittata* (C. L. Koch, 1846)	√	√	√	√
28	*Plexippus paykulli* (Audouin, 1826)	√	√	×	×
29	*Telamonia dimidiata* (Simon, 1899)	√	√	×	×
VII.	**Family- Sparassidae**				
30	*Heteropoda bhaikakai* (Patel and Patel,1973)	√	√	×	×
31	*Heteropoda venatoria* (Linnaeus , 1767)	×	√	×	×
32	*Olios spe.*	×	×	√	×
VIII.	**Family- Thomisidae**				
33	*Thomisus pooneus* (Tikader,1865)	×	√	√	×
34	*Thomisus spe.I*	×	√	√	×
35	*Thomisus spe. II*	×	×	√	×
36	*Xysticus bharatae* (Gajabe and Gajabe, 1999)	×	√	×	×
37	*Xysticus tikaderi* (Bhandari and Gajabe,2001)	×	×	√	×
38	*Xysticus spe.*	×	×	√	×
IX.	**Family- Tetragnathidae**				
39	*Lucauge decorata* (Blackwall, 1864)	√	√	√	√
40	*Tetragnatha spe.*	×	√	×	×

X.	**Family- Uloboridae**				
41	*Uloborus spe.*	√	√	×	×

(Sign √= presence of spider species and Sign × = absence of spider species)

Table No.03 Diversity Indices

Sr. No.	Agricultural Habitats	Shannon Diversity Index	Margalef's Index	Pielou's Evenness Index
01	Banana	3.178	7.237	1
02	Sugar- apple	3.434	8.736	1
03	Sorghum	3.178	7.237	1
04	Vineyard	2.485	4.427	1

V. CONCLUSION:

A total of 41 species of agrobiont spiders were recorded from four different agricultural habitats. The present study shows the diversity of agrobiont spider was higher in Sugar- apple followed by the Banana and Sorghum while it was least in the Vineyard agricultural habitat. This is because of the farmers of this area spreads more pesticides in the vineyard while pesticides are not used in Banana, Sugar- apple and Sorghum crops. The overuse of pesticide also affects the population of prey of the spider leads to the declination of spider diversity. The further research is needed to assess the impact of pesticides utilization in the agro-ecosystem on the deterioration of spiderdiversity in agro-ecosystems.

VI. ACKNOWLEDGMENT

Authors are thankful to the Incharge Principal and Head department of Zoology, Balwant College, Vita for providing laboratory facilities.

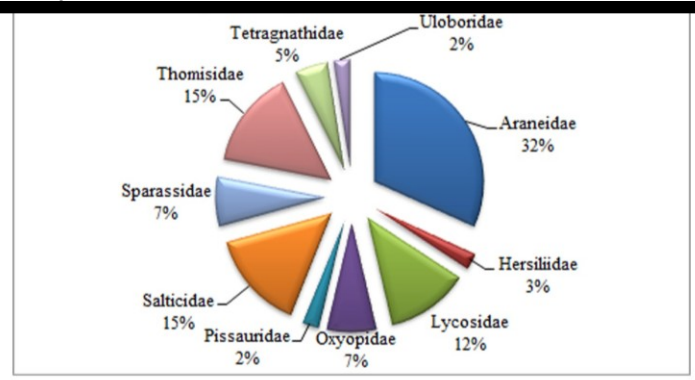

Fig. No.01- Abundance of Spider Families in four different agro- ecosystems

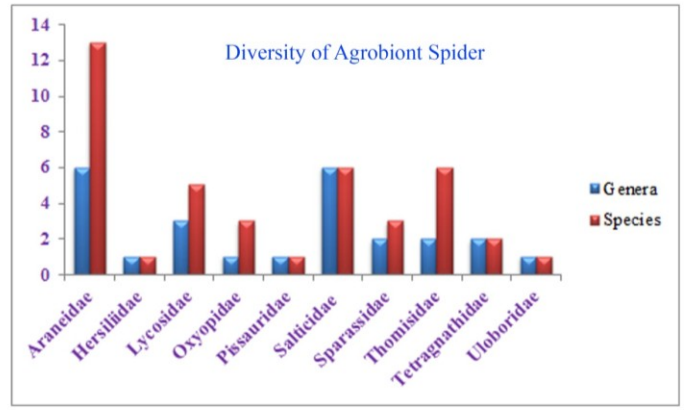

Fig. No.02- Number of genera and species of agrobiont spiders recorded four different agro- ecosystems

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EVALUATION OF WATER QUALITY STATUS OF LAKES OF TASGAON TEHSIL, DIST. SANGLI (M.S.) INDIA

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ABSTRACT: *Water is an essence of life every living thing, all living things on earth are totally dependent on water. Nowadayswater quality is affecting due to the pollution, industrialization, and overuse of chemical pesticides, fertilizers, etc. The present study reveals that the influence of environmental parameters and anthropogenic activities on water quality at different lake regions of Tasgaon Tehsil Dist. Sangli (M.S.) India, namely, Siddhewadi, Anjani, Agrani (Gavhan), Bastawade and Gaurgaon (Balgavde) on the basis of water quality index. The present Water quality index was determined for a period of one year from October 2017 to November 2018. Water quality index was determined on the basis of various physicochemical parameters like Temperature, pH, Total alkalinity, acidity, Total hardness (Ca & Mg), Chlorides, Total dissolved solids (TDS), Chemical oxygen demand (COD) and Biological oxygen demand (BOD). All the recorded parameters from different lakes were found fluctuation and within the desirable limits of tolerance as computed by WHO.*

Keywords: *Physicochemical, Water, Tasgaon, BOD, WHO.*

I. INTRODUCTION

Water is the prime source of all biological lives for their survival and growth. Without water, there would be no life on earth.Water plays an essential role in human life.The quality of water is of vital concern for mankind since it is directly linked with human welfare.Water is utilized for the domestic purpose, for industrial applications, agriculture purpose, as well as for inland fishery. Water Quality Parameters is one of the most effective tools to communicate information on the quality of water to concerned citizens. It gives the public general idea of the possible problems with water in a particular region.

Water quality has become a major global concern due to increasing human developmental activities. The present study area has come under the east region of Tasgaon tehsil where the vineyard is one of the major cash crops. More than 30 gm. chemical pesticides per hectare are spread on grapes by the farmers of this Tehsil (Kale, 2016). Most of the pesticide is mixed in surrounding water bodies this produced a high risk of side effect on non-target species like human and long-lived residues in the environment. Therefore, it is desirable to control the intake of these potentially toxic chemicals from drinking water because the intake from other sources which are food or air may be difficult to avoid. The aim of present study was to evaluate thephysicochemical status of water and to create the awareness among the peoples of different villages surrounding this lake.

II. MATERIALS AND METHODS

Study Area:

The water samples were collected from different lakes of Tasgaon tehsil namely, Anjani, Agrani (Gavhan), Bastawade, Gaurgaon (Balgavde) and Siddhewadi (Photo Plate No.01). The Siddhewadi lake present in Agrani river basin and water comes in them from the Agrani river and rainwater. Anjani water lake is manmade lake as constructed for the water supply to Anjani village as potable water and also supply agriculture area like grape cultivation, sugarcane production etc. This lake has embankment height of 11.73 meters and about 42.34 square kilometer catchment area. Bastawade water Lake has embankment height of 09.43 meters and about 14.34 square kilometer catchment area. Gaurgaon (Balgavde) has embankment height of 09.73 meters and about 15.67 square kilometer catchment area. Sidhewadi lake is a medium irrigation reservoir near Tasgaon tahsil in Sangli district. This reservoir was constructed during the year 1972 to 1977. This reservoir is very useful to nearby villagers of Savlaj, Siddhewadi, Waiphale, and Dahiwadi.The catchment area is 65 sq.km. A total capacity of storage is 302.95 Mcft and dead storage is 87.56 Mcft. The total length of the dam including slipway is 959 m. in which length of slipway is 216 m and it is without a gate. The dam is an earthen type, having a 19 m dam height.

Sample Collection:

The present study was carried out from month October 2017 to November 2018. The Water Samples were

collected from three different places in the morning hours between 9 to 11 am, in Polythene Bottles with established standard norms was adopted. Labels were used to prevent sample misidentification Sample preservation was done in tune with Ground.The collected water samples were brought to the laboratory of Department of Chemistry and Zoology and relevant analysis was performed.

The physicochemical analysis of water samples was carried out by the standard method prescribed by Kodarkar et al. (1998), Ragothaman and Trivedi (2002) and APHA (2012).

Statistical Analysis:

The data is analyzed and calculated the standard deviation and standard error by using R software (Ver.3.4.4.) and Microsoft Office Excel, 2010 (Table No.02).

III. RESULTS AND DISCUSSION

During the present investigation, different parameters were analyzed for one year from October 2017 to November 2018. The results are shown in Table No. 1 and Graph No. 01 to Graph No.07.

Temperature:

Temperature is an important factor which controls the chemical reactions and also plays an important role in the metabolic activities of the organism. In the present study, temperature range from a minimum of 28.5°C to a maximum of30°C (Table No.02 and Fig. No. 01). The temperature was recorded in the month of October, May, and July but the variation was never more than 4 to 5 degrees for any sample during the study period. Siddhewadi water had a lower temperature than other lake water in all three months.

pH:

During the present investigation, the fluctuation of pH was observed from 7.0 to 7.4. This may be attributed to the carbonate-bicarbonate buffer abundantly found in the soil. Anjani lake water has maximum pH 7.4 and Agrani water has minimum pH 7.0 (Table No.02 and Fig. No. 02).

Electrical Conductivity (EC):

The EC of water is determined by the concentration of ions present in it. The more the concentration of ions in the sample the more is its conductivity.In the present study conductivity varies from 0.407 X 10^{-3} to 0.599 X 10^{-3}mhos. The conductivity of Bastawade lake water is increased. This increase may be due to the effects of various religious activities performed at lake site and Anjani lake water conductivity is found to be minimum 0.407 X10^{-3}(Table No.02). Our findings are in good agreement with Dinesh Kumaret al. (2016) and Reda (2016).

Total Hardness:

In the present study total Hardness ranges from 64 ppm to 172 ppm. It is having a minimum value of 64 ppm at Anjani lake water and maximum value of 172 ppm at Agrani (Gavan) water (Table No.02 and Fig. No.03). The increase in hardness may be due to domestic activities like washing clothes, animals, vehicles etc. done at the lake site.Total hardness is 300mg/l which is the permissible limit both by BIS as well as WHO standards. Our findings are well coordinated with Basavaraja Simpi (2012), Salunkhe et al. (2015) and Reda (2016).

Total Solids (TS):

In the present study, TS varies from 0.50 to 1.74. Maximum TS (1.74) in Agrani Sample and minimum (0.50) in Bastawade lake water sample (Table No.02 and Fig. No.07).Soil erosion is a large contributor and naturally occurring rocks or minerals in the soil such as halite, NaCl, or limestone, $CaCO_3$, may also dissolve into the water, adding to the total solids.

Total Dissolved Solids (TDS):

Increased TDS may impart a bad odor or taste to drinking water, as well as cause scaling of pipes and corrosion. In the present study, TDS at different locations in Savlaj area of the lake of Sangli district is varied from 193 to 398 mg/l. The maximum value of TDS in the present study found to be highest (398) in Agrani River (Gavan) water and minimum (193) in Bastawade Lake water(Table No. 02; Fig No. 08). As the prescribed limit of TDS for drinking water is 500 mg/l, all the water samples have TDS concentration well below the prescribed limit.Similar results were obtained byShukla et al. (2013) and Patil et al. (2014).

Alkalinity:

In the present study, a total alkalinity of all the samples was found to vary from292 to 424 ppm. In view of higher total alkalinity values occur in Bastawade (424 ppm) lake water and its surrounding localities can be considered as very hard water. Minimum total alkalinity occurs in the Anjani lake water 292 ppm(Table No. 02; Fig No. 04). Higher alkaline waters are usually unpalatable and cause the bitter taste. Increase in alkalinity is noted which could be accounted for due to the local factors such as religious place, agricultural place etc.Our findings are well coordinated withManjare et al.(2010) and Shinde et al.(2011).

Acidity:

In the present study methyl, orange acidity was absent and phenolphthalein Acidity was ranged from 30 to 42 ppm. Maximum total acidity occurs in the Agrani (Gavan) river water (42 ppm) and minimum acidity in Siddhewadi lake water (30 ppm)(Table No. 02; Fig No. 05). Strong mineral acids, weak acids such as carbonic acid, acetic acid present in the water sample contributes to the acidity of water.

Chloride:

In the present study analysis of water, samples show the irregular variation in Chloride content from 62.48 to 103.66 mg/lit. The chloride content is within the permissible limit of Indian Standards also BIS & WHO (250 mg/l). Chloride content is minimum at Bastawade i.e. 62.48 ppm and maximum at Agrani (Gavhan) that is 103.66 ppm (Table No. 02; Fig No. 08).Our findings are well coordinated with Salunkhe et al. (2015).

Chemical Oxygen Demand:

The COD (mg/lit) of different in the lake area of Tasgaon tehsil is varied from 100 to 390 mg/lit. The maximum value of COD in the present study was found to be highest in Agrani lake (Gavan) water (390mg/lit.) and minimum in Anjani Lake water (100mg/lit.) (Table No. 02; Fig No. 09). High COD may be due to leaching of chemically degradable organic and inorganic waste matter from the intensely populated surrounding area. Our findings are in good agreement with Kanase et al. (2016) and Reda (2016).

Biological Oxygen Demand (BOD):

In the present study, BOD varies from 1225 to 1715. Maximum BOD occurred in Siddhewadi lake water which is 1715 mg/lit.and minimum BOD occurred Agrani (Gavan) water is 1225 mg/lit (Table No. 02; Fig No. 10). This is very high BOD value due to low COD value of all samples. Similar results were obtained by Patil et al. (2013)

Free Carbon dioxide:

In the present study free carbon dioxide varies from 8.8 to 22. Maximum free CO_2has occurred in Agrani Lake sample and the minimum is found in Siddhewadi lake sample(8.8),(Table No. 02; Fig No. 11).Carbon Dioxide is present in water in the form of dissolved gas. Surface waters normally contain less than 10 ppm free carbon dioxide, while some ground waters may easily exceed that concentration. Carbon dioxide is readily soluble in water. Over the ordinary temperature range (0-30 C) the solubility is about 200 times that of oxygen. Calcium and magnesium combine with carbon dioxide to form carbonates and bicarbonates. Similar findings were recorded by Makode (2012).

Table No. 01- Water samples collection sites of Tasgaon Tahsil, Maharashtra (India)

Sample No.	Sample station	Site of source
1	Siddhewadi	Lake
2	Agrani (Gavhan)	Lake
3	Anjani	Lake
4	Bastawade	Lake
5	Gaurgaon (Balgavde)	Lake

Table No. 02- Water quality index means and standard errors of different lakes of Tasgaon Tahsil, Maharashtra (India)

Water Quality Parameter	Siddhewadi lake	Anjani lake	Agrani (Gavan)	Bastawade lake	Gaurgaon (Balgavde)	IS & WHO water quality parameter
Temperature, ^oC	27.5± 0.15	28.1±0.07	29.5 ± 0.11	29.5± 0.06	29.5±0.1	
pH	7.2 ±0.15	7.4 ±0.08	7.0 ±0.10	7.2± 0.11	7.3± 0.04	6.5-8.5
Conductivity (mhos.cm^{-1})	0.432X 10^{-3}	0.407X 10^{-3}	0.428 X 10^{-3}	0.599 X 10^{-3}	0.432 X 10^{-3}	
Hardness (mg/lit.)	92 ±0.70	64± 1.41	172± 1.00	68 ± 1.14	124 ± 1.14	300
Alkalinity (ppm)	300 ± 1.14	292 ± 2.60	436± 2.0	424 ± 1.92	388 ± 1.41	200
Acidity (mg/lit.)	30 ± 0.98	38 ± 0.44	42 ± 0.70	32 ± 0.54	34± 0.70	
Chloride (mg/lit.)	69.43± 0.44	67.14 ±	103.17±	63.23 ± 0.67	74.67 ±	250

		0.64	0.68		0.91	
Total Solid (mg/lit.)	0.58 ± 0.02	0.96 ± 0.04	1.74 ± 0.01	0.50 ± 0.03	0.80± 0.08	
Total Dissolved Solid (ppm)	228 ± 0.70	212 ± 0.31	398 ± 0.44	193 ± 0.24	286 ± 0.57	500
Chemical Oxygen Demand (COD) mg/lit.	100 ± 0.21	200 ± 0.70	390 ± 0.94	324 ± 1.14	121± 1.00	250
Biological Oxygen Demand (BOD) mg/lit.	1715 ± 0.70	1463 ± 1.14	1225 ± 0.63	1633 ± 1.0	1470 ± 1.58	350
Free Carbon dioxide (mg/lit.)	8.8 ± 0.11	15.4 ± 0.08	22 ± 0.11	15.4 ± 0.12	11 ± 0.14	-

IV. CONCLUSION

In this study the collected drinking water samples of different lakes of east region of Tasgaon tehsil were analyzed for physicochemical parameterslike Temperature, pH, Total alkalinity, acidity, Total hardness (Ca & Mg), Chlorides, Total dissolved solids (TDS), Chemical oxygen demand (COD) and Biological oxygen demand (BOD). The result revealed that almost all the measured parameters were found fluctuation and within the desirable limits of tolerance as computed by WHO and BIS. But the Alkalinity of all sampling sites was found higher than the standard value of WHO. In addition to this, the Biological oxygen demand (BOD) of the sampling site was also found higher than the WHO guideline. In general, the present investigation found that the maximum parameters were not at a level of pollution and may not cause harmful effect to the consumers.

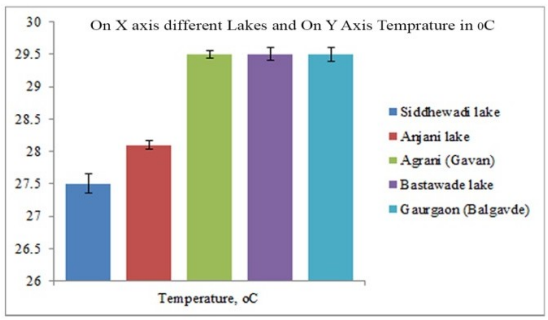

Fig . No. 01- Comparision of Temprature of different lakes of Tasgaon

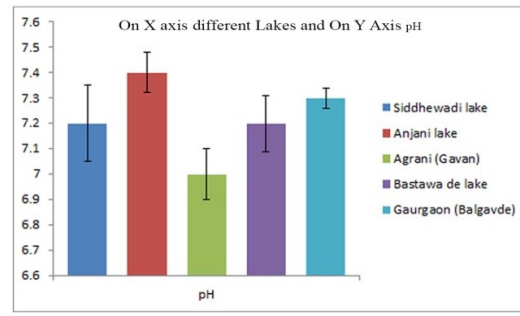

Fig . No. 02- Comparision of pH of different lakes of Tasgaon Tehsil

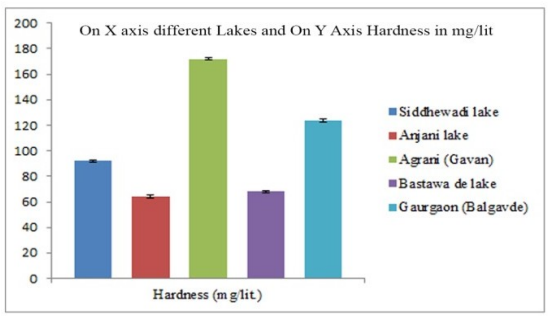

Fig . No. 03- Comparision of Hardness of different lakes of Tasgaon Tehsil

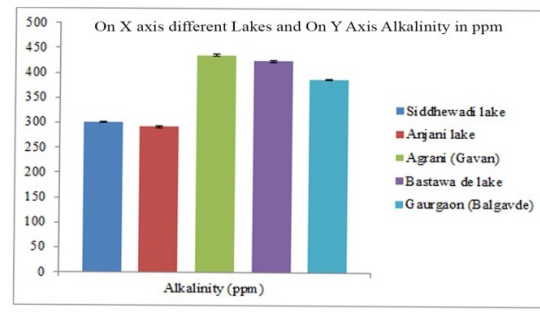

Fig . No. 04 - Comparision of Alkalinity of different lakes of Tasgaon Tehsil

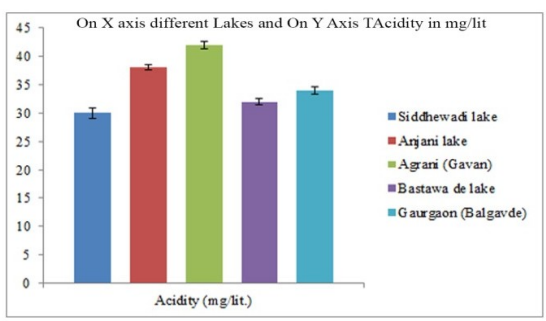

Fig . No. 05- Comparision of Acidity of different lakes of Tasgaon Tehsil

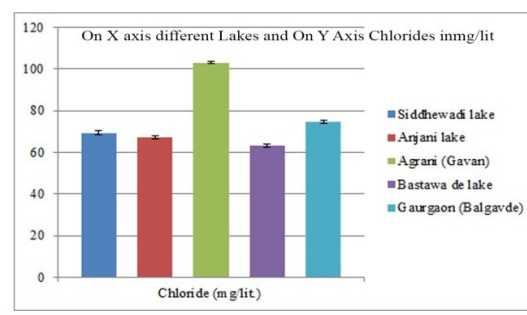

Fig . No. 06- Comparision of Chloride of different lakes of Tasgaon Tehsil

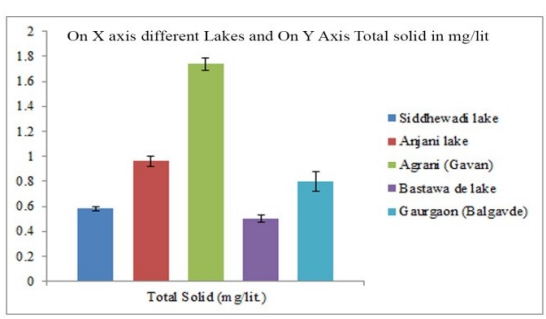

Fig . No. 07- Comparision of Total Solids of different lakes of Tasgaon Tehsil

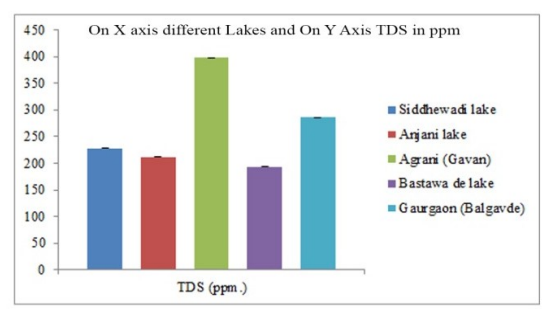

Fig . No. 08- Comparision of Total dissolve solid of different lakes of Tasgaon

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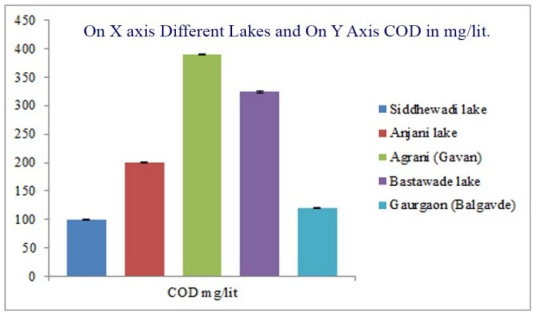

Fig. No. 09- Comparison of COD of different lakes of Tasgaon tehsil

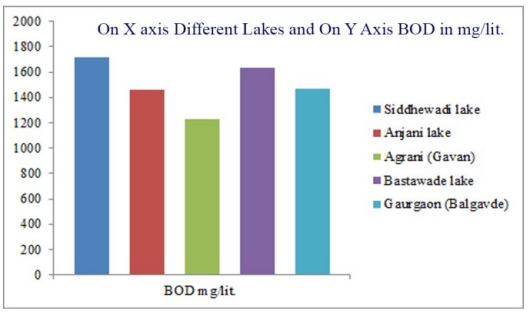

Fig. No. 10 - Comparison of BOD of different lakes of Tasgaon tehsil

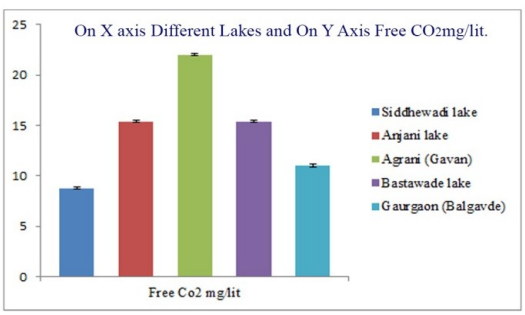

Fig. No. 11- Comparison of Free CO_2 of different lakes of Tasgaon tehsil

Photo Plate No 01:- A. Study area Tasgaon Tehsil B. Siddhewadi Lake C. Anjani Lake D. Agrani (Gavan) E. Bastawade Lake F.Gaurgaon Lake

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DIVERSITY AND ABUNDANCE OF BUTTERFLIES (INSECTA: LEPIDOPTERA) IN AND AROUND VITA CITY, DISTRICT SANGLI, (M.S.) INDIA

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ABSTRACT: *The present paper deals with a study of the diversity and abundance of butterflies in and around Vitacity (M.S.), India. The study was conducted during Jun. 2017 to Dec.2018. A Total 33 species of butterflies belonging to 24 genera and 05 families, namely, Hesperiidae, Lycaenidae Nymphalidae, Papilionidae and Pieridae were recorded from different habitats, urbanized habitat i.e. Vita city (Site I) and less urbanized habitat (Forest and agriculture) (Site II). The family Nymphalidae (39.39%) was found abundant and family Papilionidae (12.12%) was found least abundant among all the families. The diversity index has shown that the species diversity is maximum in the site II (Shannon- index 3.342) as compared to the site I (Shannon- index 2.839). The present study may provide basic data for the study of butterfly diversity of Sangli District. The results were analyzed with presently available literature.*

Keywords: *Vita, Butterfly, Diversity, Habitat, Shannon Index*

I. INTRODUCTION

Butterflies are classified into class Insects and order Lepidoptera,which is the second largest order in the animal kingdom. Theword Lepidoptera means `Scale wings`(Greek; Lepis- scale; Pteron-wing).They are one of the most beautiful and striking species of insect on the earth and they are playing a very crucial role in the ecosystem as well as human health. They are commonly referred to as "insects of the sun" with their eye-catching color and delicate charisma (Haroon, 2016). They have been admired for centuries for their physical beauty and behavioral display (Arya and Chaudhari, 2014). The presence of butterflies indicates the good condition of an ecosystem (Aluri and Rao 2012). They have always enthralled common man because of their daintiness and beauty (Arya et al., 2014). Recently many hospitals were started to prepare butterfly gardens in around the hospital to maintain the healthy ecosystem around the hospitals.
India represents one of the "megadiverse" countries of the world. Out of about 25,000 species of butterflies recorded from all over the world, 1501 are from India (Gay *et al.,*1992). Northern western ghat is a hot spot of biodiversity where total 191 species of butterflies belonging to 117 genera and 06 families recorded (Padhey *et al.,* 2013). The diversity of butterflies from Sangli district which comes under the study area is not studied well hence the present attempt was carried out. The aim of present study is to find out the current status of butterflies in and around Vita city and to prepare a checklist of butterflies of this region for the purpose of conservation of butterfly species present in this area.

II. MATERIALS AND METHODS

2.1 Materials: Study area:

Vita city is one of the big city of Khanapur tehsil which is situated at Sangli district of Maharashtra state. It is commonly called "The City of Gold". It is situated on two state highways *viz*., Guhagar- Bijapur, and Ahmednagar Sangli. Vita city is situated on $17^0 16'16''$N and$74^0 32'16''$E. It belongs to Paschim Maharashtra region. The total land area covered by Vita is 55.3 $km^{2.}$ [A] Total population of Vita city is 48,289. The average temperature of Vita city ranges from 14 to 40^oC. A Climate of the Vita is dry and average rainfall is 80 -120 mm.

Vita city is surrounded by well agriculture land, grassland area, several manmade biodiversity parks and water bodies which gives a good habitat for the development of butterflies.

2.2 Data Collection:

The butterfly survey was carried out from the Jun. 2017 to Dec. 2018 from two sampling sites Vita city i.e. urbanized habitat (Site I) and surrounding Agriculture land, grasslands and biodiversity park i.e. least urbanized area (Site II). Site II has selected approximately three- kilometer area of around the Vita city. The observations were carried out by a weekly visit to Site I and Site II during morning and evening time. The Pollard Walk method was used for observing butterflies that is walking along the fixed paths while

recording these species (Pollard 1977,1993). Species were photographed by using Nikon D5300 camera (lens 18-55mm) and identified in their natural habitat. When it was difficult to assess, then specimen were captured for their further identification and after identification, they were released in their natural habitat immediately.

The collection of specimens is carried out by using a sweep net. The specimens are observed and some are collected from herbs, shrubs and near ponds from study sites. The identification was done by the standard identification key prescribed by Bhakare and Ogale (2018) in the book of Butterflies of the Western Ghats and Kasambe (2018) ebook of Butterflies of Western Ghats.

2.3. Statistical Analysis:

4. **Diversity is calculated by using Shannon-Weaver index (H`).**

Formula $H` = - \Sigma Pi \ln Pi$

Where,

Pi = S/N

S = Numbers of individuals of one species

N = Total number of all individuals

In = Logarithm to base e.

5. **Evenness of the species is calculated by using Pielou's evenness index**.

The evenness index ranges from zero to one, where zero signifying no evenness and one as complete evenness.

Formula $e = H/ \ln S$

Where,

H = Shannon- Weaver index.

III. RESULTS

A total of 401 individuals of 33 species of butterflies belonging to 24 genera and 05 families were observed in and around Vita city (Table No. 01 and 02). The family Nymphalidae (39.39%) was most abundant family represents 08 genera and 13 species followed by Lycaenidae (18.18%) represents 05 genera and 06 species, Pieridae (18.18%) represent 05 genera and 06 species, Hesperiidae (12.12%) represents 04 genera and 04 species and Paplionidae (12.12%) represents 02 genera and 04 species (Table No. 01 and Fig. No. 01 and 02). The *Eurema brigitta* was found the abundant species (7.98%) while*Megisba malaya*(0.24 %) was found scarce in both site, Site I and Site II (Table No. 02). The Site II was recorded highest species diversity (Shannon Index-3.342) than Site I (Shannon Index-2.839) (Table No.03). The species evenness index was being highest in Site II (Pielou's Evenness Index - 0.8833) whereasit was found less at the Site I (Pielou's Evenness Index -0.8145). These results indicate that the diversity and evenness of butterflies were affected in Site I due to the loss of habitat and increased human activities like construction of buildings, roads, towers, etc.

IV. DISCUSSION

Thirty -Three species of butterflies were recorded over the period one year. Open scrub, agriculture land and grassland area (Site II), the least disturbed area, were found to have the highest species richness as comparing urbanized habitat (Site I), the most disturbed. These results can be attributed to the presence of the host and larval plant species, whose occurrence impacts the distribution of butterflies. Tiple et al. (2007). Similar results were recorded by several researchers,Harsh (2014), studied the diversity of butterfly species in three different habitats of the IIFM campus Bhopal and recorded; the open scrub has the greatest species number with 52 species while urbanized habitat ranks lowest with 44 species. Gaikwad et al. (2015) recorded 35 species of butterflies from Phaltan region, district Satara (M.S.). They found the Lycaenidae was the largest family followed by Nymphalidae, Hesperiidae, Pieridae, Papilionidae and Danaidae family. Gupta (2018) recorded 15 species of butterflies belonging to 11 genera and 04 families fromthe campus area of Amolokchand Mahavidyalaya Yavatmal, Maharashtra, India. More et al. (2016) recorded 52 species of butterflies belonging to 36 genera and 05 families.

Our results are in good agreement with the findings of Padhyeet al. (2006),Ramesh *et al.* (2010),Tiple et al. (2011), Kunte et al.(2000), and Patil et al. (2014).Apart from these, the differences observed may be because of climatic and geographical variation of the place.

Table No. 01 Number of Families, Genera, and Species of butterfly recorded In Site I (Urbanized) and Site II (least Urbanized).

Sr.No.	Family	Genera	Species	Species %
01	Hesperiidae	04	04	12.12 %
02	Lycaenidae	05	06	18.18 %
03	Nymphalidae	08	13	39.39 %
04	Papilionidae	02	04	12.12 %
05	Pieridae	05	06	18.18 %
Total		**24**	**33**	**100 %**

Table No. 02: List of Butterfliesrecorded in the study area (SI-Vita City, SII-Agriculture land)

Sr. No.	Family and Common Name	Scientific Name	Abundance
	Family - Hesperiidae		
01	Banana Skipper	*Erionota thorax*	1.49 %
02	Common Small Flat	*Sarangesa dasahara*	0.99 %
03	Conjoined Swift	*Pelopidas conjuncta*	0.49 %
04	Indian Skipper	*Spialia galba*	1.74 %
	Family - Lycaenidae		
05	Gram Blue	*Euchrysops cnejus*	2.74%
06	Grass Jewel	*Freyeria trochylus*	2.49 %
07	Lesser Grass Blue	*Zizina otis*	0.74 %
08	Malayan Owl	*Megisba malaya*	0.24 %
09	Plains Cupid	*Chilades pandava*	0.99 %
10	Stripped Pierrot	*Tarucus nara*	3.74 %
	Family – Nymphalidae		
11	Anomalous Nawab	*Polyura agraria agraria*	2.24 %
12	Blue Pansy	*Junonia orithya swinboei*	2.49 %
	Family -Nymphalidae		
13	Blue Tiger	*Tirumala limniace*	3.49 %
14	Common Crow	*Euploea core core*	5.73 %
15	Common Evening Brown	*Melanitis leda leda*	2.49 %
16	Chocolate Pansy	*Junonia iphita pluviatalis*	2.49 %
17	Danaid Eggfly	*Hypolimnas misippus*	6.23 %
18	Dark Evening Brown	*Melanitis phedima varah*	2.24 %
19	Great Eggfly	*Hypolimnas bolina jacintha*	4.98 %
20	Lemon Pansy	*Junonia lemonias vaisya*	1.49 %
21	Plain Tiger	*Danaus chrysippus chrysippus*	7.98 %
22	Peacock Pansy	*Junonia almana almana*	2.49 %
23	Yellow Pansy	*Junonia hierta hierta*	0 %
	Family - Papilionidae		
24	Common Mormon	*Papilio polytes*	4.73 %
25	Common Jay	*Graphium doson eleius*	4.48 %
26	Rose Mormon	*Papilio polymnestor*	5.48 %
27	Lime Butterfly	*Papilio demoleus*	1.99 %
	Family - Pieridae		
28	Common Jezebel	*Delias eucharis*	2.74 %
29	Common Emigrant	*Catopsilia pomona*	0.99 %
30	One Spot Grass Yellow	*Eurema andersoni*	2.49 %
31	Plain Orange Tip	*Colotis aurora*	2.49 %
32	Small Grass Yellow	*Eurema brigitta*	7.98 %
33	Yellow Orange Tip	*Ixias pyrene*	3.24 %

Table No.03 Diversity Indices

Sr. No.	Site No.	Shannon Diversity Index	Pielou's Evenness Index
01	Site I (Urbanized habitat)	2.839	0.8145
02	Site II (Less Urbanized)	3.342	0.8833

V. CONCLUSION:

The present study was clearly revealed that the diversity of butterflies is higher in Site II because of this is less human-disturbed area and having more number of host plants, grassland area, and water bodies which is favorable habitat for the development of butterflies. The diversity is found in a site I (Vita City) is less because of loss of habitat and due to increased human activities, like the construction of buildings, roads, towers, etc. Further research is needed to assess the impact of urbanization on the deterioration of butterflydiversity.

VI. ACKNOWLEDGMENT

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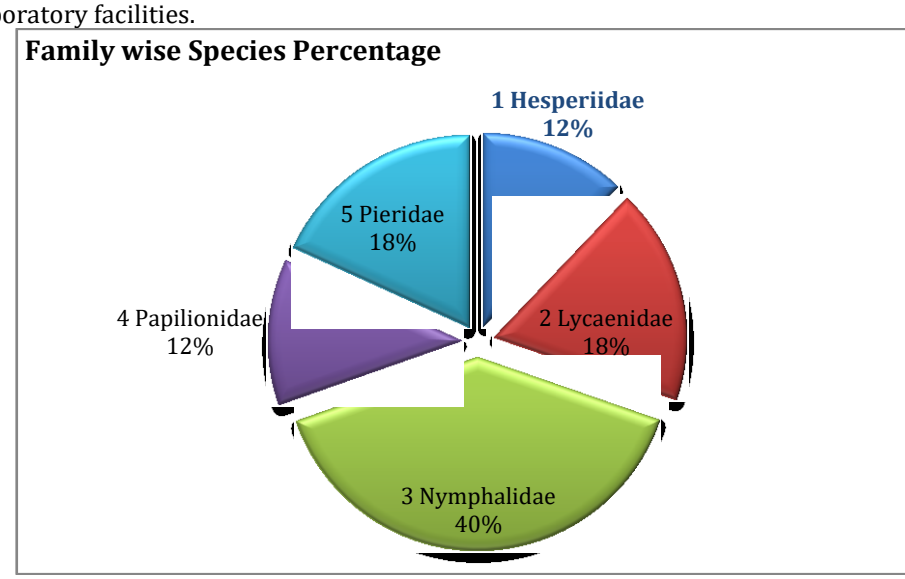

Fig. No.01- Abundance of butterfly Families in Site I and Site II

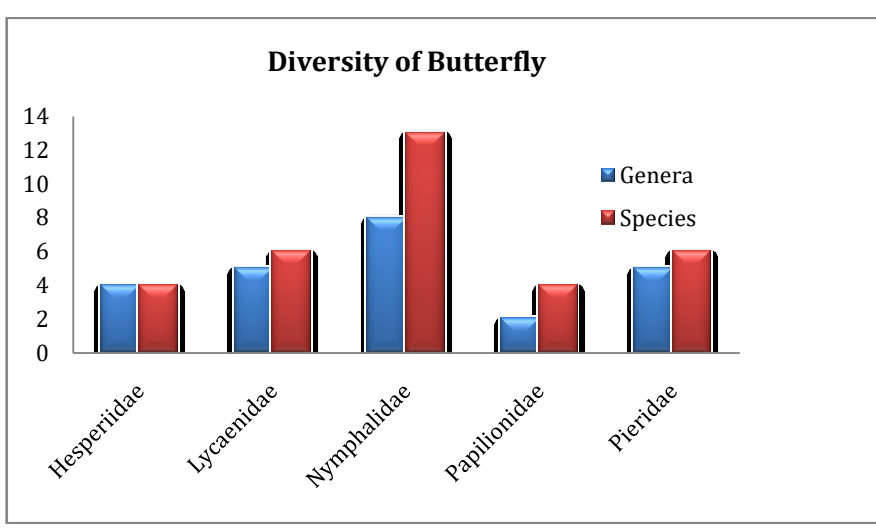

Fig.No.02- Number of genera and species of butterflies recorded in Site I and Site II

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FAUNAL ASSESMENT OF LONG HORNRD GRASSHOPPERS FROM AGRICUTURAL LANDS OF ARID REGION OF SATARA DISTRICT OF MAHARASHTRA

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ABSTRACT: *The Orthopteran insects are mostly well known, as their inhabitants in our surroundings, gardens, farms, grasslands forests etc. and they directly and indirectly impacts on society, biology, ecology, flora, and fauna.The Tettigoniids or long-horned grasshopper belongs to family Tettigoniidae of order Orthoptera.In the present investigation 7 species of Tettigoniidae were recorded under 7 genera belongs to two sub families. The record of 7species is highlights the importance of the study.If long term study carried out many other unexplored species will come to know.*

Keywords: *Orthoptera, Tettigoniidae, Fauna, Arid region.*

1. INTRODUCTION

The order Orthoptera are the group of insects which are mostly well known, as their inhabitants in our surroundings, gardens, farms, grasslands and forests. They directly and indirectly impacts on society, biology, ecology, flora, and fauna. They also playing role as a biological indicator of Environmental changes, habitat destruction, pollution.Also their presence give an idea of present biota which depend on them and on which they depend. Their diversity and distribution study will give an idea to put local biota, flora and fauna in the specific frame. Earlier Orthepteran diversity workers Kirby (1914) and Chopard (1969) estimate the satisfactory diversity in Indian subcontinent while latter some workers Bhowmik (1985a, 1986), Shishodia and Hazra (1986), Mandal et al. (1999, 2007), Shishodia (1991, 1997, 2000a, b), Hazra et al. (1993, 1995), Vasanth (1993), Barman (1993, 1995, 2000, 2003), Shishodia & Tandon (2000), Sigfrid and Shsihodia (2000), Dey & Hazra (2003), Shishodia et al. (2003), Mandal & Yadav (2007), Gupta et al. (2008) gives their contribution to exploration of diversity. While capacious contribution given to the Orthoptera fauna of Maharashtra has been given by Nadkerny (1965), Vasanth (1980), Shishodia (1991), Sharma et al. (1999), Kulkarni & Sharma (2004) and Kulkarni & Shishodia (2004, 2005),Gaikwad et al. (2016) etc.

The Tettigoniids or long-horned grasshopper belongs to family Tettigoniidae of order Orthoptera. From the Maharashtra state 18 species of Tettigoniidae recorded (Chandra and Gupta 2012) belongs to 14 genera in the Fauna of Maharashtra, State Fauna Series. Gaikwad et al. (2016) recorded 17 species under 16 genera of Tettigoniidae reported from Radhanagari Wildlife Sanctuaryin which six species were first time reported from Maharashtra totals 24 species.

The specimens of Tettigoniidaeas they are mostly nocturnal were collected during evening hours 4 pm to 6 pm using insect sweeping net and in night hrs (7 pm to 11 pm) during June 2018- October 2018) with the help of the torch and at light sources by hand picking method. They were killed and preserved as wet preservation method. They were identified with the help of literature of Srinivasan (2012),http://Orthoptera.SpeciesFile.org,and Gaikwad et al. (2016).

In the present investigation 7 species of Tettigoniidae were recorded under 7 genera belongs to two sub families. Earlier from the Satara district diversity of Tettigoniidae was never been done therefore present study provides a baseline data for further research.

Order: Orthoptera
Suborder: Ensifera
Infraorder: Tettigoniidea
Superfamily: Tettigonioidea
Family: Tettigoniidae
Subfamily: Phaneropterinae
Genus *Ducetia* Stal, 1874
1.*Ducetiajaponica* Thunberg, 1815
1815 *Locusta japonica* Thunberg. Mem. Acad. Imp. Sci. St. Peterburg 5:282
Material Examined:1♀ Jashi, Satara Dist, 18. x. 2018.

Distribution: India: Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Himachal Pradesh, Jammu & Kashmir, Madhya Pradesh, Maharashtra, Meghalaya, Nagaland, Odisha, Rajasthan, Sikkim, Tamil Nadu, Uttarakhand, Uttar Pradesh and West Bengal;

Remark: This species is associated with grass. It is found in plains as well as agricultural lands. Adults are common during the months of December and January.

Genus *Elimaea* Stal, 1874

Genus *Letana* Walker, 1869

2. *Letana megastridula* Ingrisch, 1990

1990*Letana megastridula* Ingrisch. Entomologica Scandinavica 21(3):258.

Material Examined: 1♂ Mardi,Satara Dist, 10. viii. 2018.

Distribution: India: Bihar, Chhattisgarh, Himachal Pradesh, Maharashtra and Tamil Nadu. **Remarks:** This species is generally found on grass. Adults are prevalent in the months of October and November. It is easily recognized by black or reddish dots on body and legs.

Genus: *Trigonocorypha*, Stal, 1874

3. *Trigonocorypha unicolor* Stoll, 1787

1787*Gryllus (Tettigonia) unicolor*Stoll. Représentation exactement colorée d'après nature des spectres ou phasmes, des mantes, des sauterelles, des grillons, des criquets et des blattes 13.

Material Examined: 1♂ Dahiwadi, Satara Dist.,17.viii.2018.

Distribution: India: Andaman & Nicobar Islands, Karnataka, Maharashtra, Meghalaya, Odisha, Rajasthan, Tamil Nadu and West Bengal; Java and Sri Lanka.

Remark: Large species, easily identified by notable green coloured wings with yellow veins.

Subfamily: Pseudophyllinae

Genus *Sathrophyllia* Stal, 1874

4. *Sathrophyllia rugosa* (Linnaeus, 1758)

1758*Gryllus (Tettigonia) rugosus* Linnaeus. Systema Naturae per Regna tria naturae (10th ed.) 1:430.

Material Examined: 1♂ 1♀ Mardi, Satara, Dist, 07.viii.2018;1♂ Jashi, Satara Dist, 15.viii.2018.

Distribution: India: Karnataka, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Sikkim, Tamil Nadu & West Bengal; Java, Sri Lanka and Sumatra.

Remarks: This species is associated with tall grass and trees. Maximum population observed in the month of October.

Genus *Paramorsimus* Beier, 1954

5. *Paramorsimus oleifolius* Fabricius, 1793

1793*Locusta oleifolia* Fabricius. Supplementum Entomologiae Systematicae 2:35.

Material Examined: 1♂ 1♀ Mardi, Satara, Dist, 07.viii.2018;1♂ Jashi, Satara Dist, 15.viii.2018.

Distribution: India: Maharashtra, Odisha and Tamil Nadu.

Remark: This species id reported from only above three states which states that the distribution of this species is not very well known. Therefore the report of this species from the region is important.

Subfamily Conocephalinae

Genus *Conocephalus* Thunberg, 1815

6. *Conocephalus (Anisoptera) maculatus* (Le Guillou 1841)

1841*Xiphidion maculatus*Le Guillou. Revue et Magasin de Zoologie 294.

Material Examined: 2 ♂ 1 ♀ Mardi, Satara, Dist.19.viii.2018, 1♂ Jashi, Satara Dist. 20. viii. 2018.

Distribution: India: Andaman & Nicobar Islands, Arunachal Pradesh, Chhattisgarh, Himachal Pradesh, Jammu & Kashmir, Kerala, Madhya Pradesh, Maharashtra, Manipur, Mizoram, Nagaland, Odisha, Sikkim, Tamil Nadu, Tripura, Uttarakhand and West Bengal.

Remarks: Maximum population observed in the month of November. This species can easily be recognized by large dark spots on tegmina. This species is carnivorous.

Genus *Euconocephalus* Karny, 1907

7. *Euconocephalus incertus* (Walker, 1869)

1869 *Conocephalus incertus* Walker, F. Catalogue of the Specimens of Dermaptera Saltatoria in the Collection of the British Museum 2:320.

Material Examined: 1 ♂ Mardi, Satara, Dist.19.viii.2018.

Distribution: Andaman and Nicobar Islands, Chhattisgarh, Madhya Pradesh, Maharashtra, Meghalaya, Nagaland, Orissa, Pondicherry, Rajasthan, Sikkim and West Bengal.

Remark: The maximum population observed in the month of December and January. This species is associated with hilly region among dry long grasses.

Earlier from the Satara district diversity of Tettigoniidae was never been done therefore present study provides a baseline data for further research. The present exploration 7 species from the region is satisfactory as compared to 18 species from Maharashtra (Chandra and Gupta 2012) and 11 species from Radhanagari Wildlife Sanctuary which highlights the importance of the study.If long term study carried out many other unexplored species will come to know.

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EFFECT OF PARSLEY ON SUBMANDIBULAR GLAND OF NATURALLY AGED MALE MICE

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ABSTRACT: *The aging is generally characterized by the declining in ability to respond to stress, increasing homeostatic imbalance and increased risk of diseases like, Xerostomia, Cancer and Syndrome like Sjogern's. These diseases promote free radical formation in the cell. Free radicals formed due to various reasons are scavenged by antioxidants. Antioxidants are present in large amount in plants like Petroselinum crispum, Bacoppa moniera, Withania somnifera. In this investigation, Parsley(Petroselinum crispum.), corrected the histological structure of submandibular gland in Parsley receiving naturally aged male mice. Histological technique Haematoxylene Eosine (H+E) was used in this study. In present investigation, three groups (control, naturally aged and parsley receiving) were made. The cross section of submandibular gland of adult male mice (control- Age 42 ± 0.6) show well formed acini (AC), convoluted granular tubules and ducts (dc) were clear and darkly stained. In naturally aged mice (Age 60 weeks) submandibular gland, number and size of acini were reduced. Organization of acini and granular convoluted tubules with respect to one another was lost. The structure of submandibular gland of parsley receiving mice nuclear staining reactivity of both the granular cells (GC) and acini (AC) was increased as well as architecture of acini became normal.*

Keywords: *Aging, Submandibular gland, Parsley, Haematoxylene, eosin.*

I. INTRODUCTION:

Salivary glands are readily accessible and well characterized. Therefore they are useful tool for the study of the normal aging process and the impact of stress on organ reserve and secretary function (Baum *et al* 1992). A common generalization associated with aging is that Salivary gland function is altered (Storer, 1978) and diminished out put results in dental carries, altered mucosal integrity and impaired taste and agglutination. Salivary gland dysfunction has been traditionally attributed to old age and complains of dry mouth (xerostomia) are most frequent in elderly experiences, the deleterious consequences of Salivary hypofunctions. (Narhi *et al* 1999, Thomson *et.al*. 1999).

Salivary gland tissue revealed age associated decreases in the number of acini and volume of convoluted granular tubules, while salivary gland production remains age stable in healthy, non medicated adults (Ship *et al* 1995; Ghezzi *et al* 2000). Here secretory reserves in salivary glands have been hypothesized to account for loss of cells in normal aging in the presence of preserved functional capacity in older adults (Scott *et al* 1987).

The Salivary glands are the collection of somewhat dissimilar structure in the mouth. There are three pairs of major Salivary glands; they are referred as the Sub mandibular also called as Sub maxillary, sublingual gland and parotid gland. They produce and secrete common juice saliva in the mouth.

In Submandibular glands there are acinar cells, together formed called acini, which secrete mainly glycoproteins. Acini are surrounded by myoepithellial cells and supported by parenchyma. There are various ducts as intercalated ducts, striated duct and granular ducts, which are also called as granular convoluted tubules. Granular ducts which have long been interesting because these ducts have been considered to be the site of formation of many enzymes like Kallikrein ,(Hosima *et al.* 1977); Proteases (Sreebny and Meger 1964, Riekkinen and Niemi 1968, Bhoola *et al* 1973); nerve growth factor (Goldstein and Budman 1965, Ellison 1967, Hendry and Wersen; 1973, Schwah *et al* 1967), epidermal Growth factor (Cohen 1962, Young and Van Lennep 1978) and various mesodermal growth factor (Weimer and Haraguchi 1975). Parsley is considered to be one of the highest sources of flavonol glycosides (Kreuzaler and Hahlbrok 1973). Parsley has been shown to possess remarkable anti inflammatory, antioxidant and anti carcinogenic property (Patel *et al*2007).

II. MATERIALS AND METHODS:-

For the histology of submandibular gland tissue was fixed in 10% neutral buffer formalin fixative for 24 hours. The tissue was washed in running tap water for 24 hours dehydrated through alcohol grades,

cleared in xylene and embedded in paraffin. The sections was at a thickness of 6 microns and stained with Eosin and Haematoxylene (H+E).

Naturally aging group:-

- **Control:-**
  Adult male mice (Age 23 week's olds and weight 42 ± 0.6 gm) was treated as control mice,
- **Naturally aged group:-**
  Naturally aged mice (Age 76 week's old and weight 38.66 ± 1.032 gm) was used.
- **Parsley receiving old mice:**
  Old male mice (Age 76 week's old weight 38.66 ± 1.032 gm) was injected parsley 40 mg / kg body weight / day subcutaneously for 20 days.

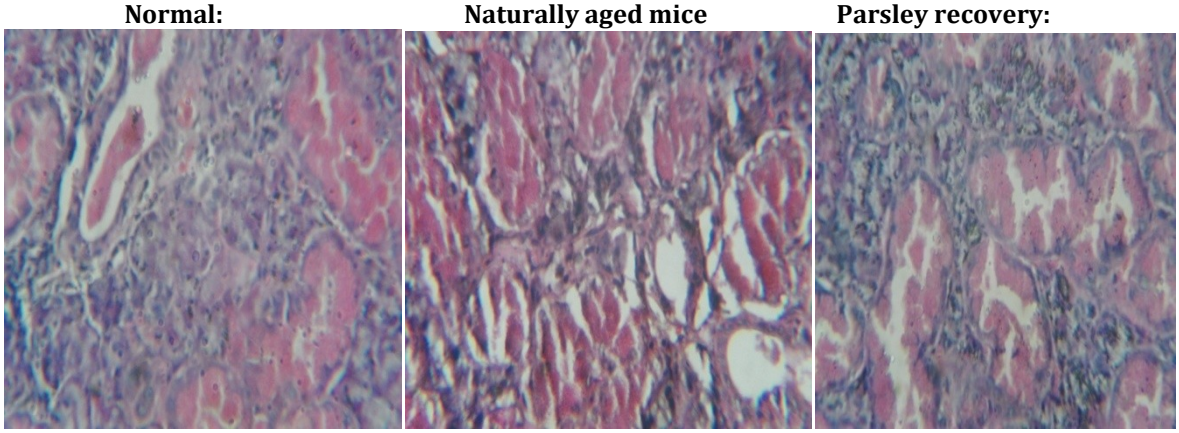
Method

Histology:-

Submandibular gland of each group was used for the histological study 10% neutral buffer formalin fixed. Paraffin embedded sections were stained with haematoxylene and Eosin (H+E).

III. OBSERVATION AND RESULT:

Normal: **Naturally aged mice** **Parsley recovery:**



The cross section of submandibular gland of adult mice (Age 42 ± 0.6) it's structure were having well formed acini (AC) and convoluted granular tubules and ducts (dc) were clear and darkly stained. In naturally aged mice (Age 60 weeks) submandibular gland, number and size of acini were reduced. Organization of acini and granular convoluted tubules with respect to one another was lost. Ducts (D) were disorganized cell layer of the duct was thin, reduced staining reactivity of acini (AC) and GC was lost and also their integrity depicts the structure of submandibular gland of parsley receiving mice nuclear staining reactivity of both the granular cells (GC) and acini (AC) was increased as well as architecture of acini became normal, but not of the granular convoluted tubules.

IV. DISCUSSION:

Major salivary glands are characterized by the presence of numerous secretary units of acini, a peculiar duct system; myoepithellial cells in addition convoluted granular tubules system, in the submandibular gland under goes changes with age. There is the reduction in the volume of acini with concomitant increase in the ductal system during aging. (Scott *et al* 1986; Kim and Allen 2005) In parenchyma vacuoles are formed, nuclei are destained (Scott 1977). Age induced variation and reactive changes, also include oncocyte proliferation. Fatty infiltration squamous and mucous metaplasia, hyperplasia and atropy (Gresik and Azmita 1990).

The purpose of this study was to utilize salivary gland to examine the effect of dietary antioxidant on stressed salivary glands where mice were treated with galactose, galactose leads to glycation and generate free radicals (Song *et al* 1999, Deshmukh *et al* 2004) which affect the organ system showing changes those are taking place in normal aging. Dietary antioxidants are polyphenolic compounds that are ubiquitously present in foods of plants origin. They may have beneficial to health. The sufficient intake of the dietary antioxidant like quercetin, myricetin and kampferol was immensely associated with subsequent decrease in coronary heart disease in some but not all prospective epidemiological studies (Hoffman 1999).

Sufficient supply with antioxidants from diet might help to prevent the occurrence of pathological changes associated with oxidative stress (Giugliano 2000)

V. CONCLUSION:

Now days, there is common consensus for the use of natural antioxidants. Flavonoids are natural and strong multifocal antioxidants, widely used flavonoids like quercetin rich in plants like cabbage, potato, red radish, beet, onion, lettuce and Parsley. Among these parsley is the rich source of quercetin. Present finding indicate Parsley may be useful in treating submandibular gland disease.

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A STUDY OF WATER QUALITY STATUS OF RIVER KRISHNA DISTRICT SANGLI, SOUTH MAHARASHTRA

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ABSTRACT: *Physico -chemical characteristics of river Krishna district Sangli Maharashtra was study during June to November 2018. The parameters were analyzed monthly such as PH, Temperature, colour, TDS, Conductivity and Dissolved Oxygen while sodium, carbonates, bicarbonates, sulphates, magnesium, chlorides, phosphate etc. The result reveals that the most of parameters are within permissible level when compared with WHO and ICMR.*

Keywords: *Krishna River, Physico-chemical parameters, Sangli district.*

1. INTRODUCTION

River system comprises both main course and tributaries carrying the one way flow of sediment with load of dissolved matter course and tributaries carrying the one way flow sediment with load of dissolved matter and particular phases coming from natural and anthropogenic sources (Rani *et al.*2011). Pollution parameters have been classified as physical, chemical and biological on the basis of analytical tests. Physical parameters include PH, temperature, colour, turbidity, conductivity, suspended matter, and dissolved matter. Chemical parameters include inorganic salts, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrogen, Alkalinity, Chloride Content, Hardness of water, and different heavy metal ions present in water. Biological parameters include zooplanktons, phytoplanktons and benthic macro invertebrates etc. Urbanization, industrialization and modernized agriculture has caused inevitable effects on physical characters of environment (viz. air, water and soil) which increased pollution (Kumar *et al.,*2003). The usage of water by man for survival is an important as that of fish and other aquatic animals (Boyd,1982 and King 1998).Good quality of water will produce healthier humans/ aquatic animals than one with poor water quality (Fafioye *et.al* 2005) .

Vijaya Deshpande *et al*.,(2002) also stated that It becomes important to determine the quality of water so that the suitability of water for drinking purpose, agriculture purpose and industrial purpose can be evaluated. have classified water based upon its extent of pollution. The quality of particular water is determined by its Physico-Chemical properties.

The level of harmful element is found to increasing in all rivers. So, an attempt is made to study the physico-chemical parameters of Krishna river water. In the present study the water of Krishna River at Sangli District has been analyzed. Previously Krishna river was only major water source for domestic uses. Now it has been neglected and water is considered unfit for domestic uses due to the accumulation of agricultural waste and sewage water. The main objective of the present investigation was to assess the physiochemical parameters of Krishna river water.

The main objective of study is:

1) To access the present quality of Krishna river.
2) To compare the results with WHO norms and ICMR norms.
3) To suggest methods of purification if required.

2. MATERIALS AND METHODS

STUDY AREA

The Krishna River is the second largest eastward draining, Perennial River in the Peninsular India. The River Krishna drains an area of 258,948 km^2, which is nearly 8% of the total geo-graphical area of the country. There are about 25 towns within the basin with the population more than hundred. The average annual rainfall in the river basin is about 780 mm. The wet seasons sets in by the middle of June and withdraws by the middle of October. About 90% of the rainfall occurs during the wet season (June-October)

and during the rest of the year (dry season) there is very little rainfall with no regular pattern.. The important land uses include agricultural land use (double crop – 35 %; single crop – 25 %), forests (15%), waste land (15%) and mixed land use (10%).The predominant soils in the area are sandy loams and loams. The river reach between the monitoring stations is approximately 35 km long with two tributaries joining the river. The sampling station includes nine different locations from Sangli districts of Maharashtra. Geographically located at Sangli(16° 52'N 74° 34' E), Digraj (16^{0}91'N 74^{0}52'E),, Bramnal(16^{0}88'N74^{0}37'E), Bhilwadi(16^{0}98'N64^{0}41'E), Borgoan (16^{0}65'N74^{0}55'E), Bahe (17^{0}02'N74^{0}28'E), Pundi (17^{0}09'N74^{0}44'E), Audumbar (16^{0}98'N74^{0}47'E) etc.

Study Area Map

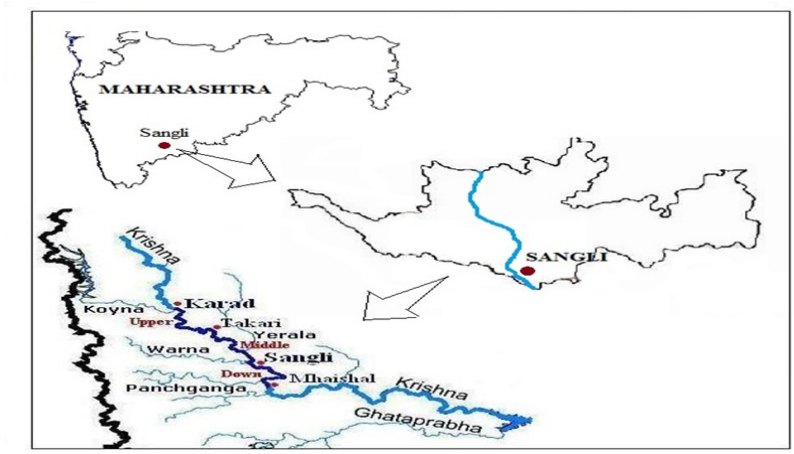


Fig.1 Map of Study Area

Sampling and analysis of water:

Study of physico -chemical characters of Krishna river water was carried out in the month of June to November 2018.For the present analysis water sample were collected in morning 9.00-10.00am. Some parameters like Temperature, pH, are directly measured at study site while other parameters were analyzed in laboratory. Air tight water bottle was used to carry water sample in the laboratory (RIRD) , Satara. Wrinklers method was followed to analyse dissolved oxygen (DO). Ca, Mg,Na, Carbonate, Bicarbonate, Chloride and Sulphate were determined laboratory as per American Public Health Association (APHA) standards.

3. RESULTS AND DISCUSSION

Total twelve physico-chemical parameters were analyzed from eight places such asBahe, Borgoan, Pundi, Audumbar, Bhilwadi, Bramnal, Digraj,Sangli. The average results are shown in Table 1.

Table 1. Average value of physico-chemical Parameters of study area during June to November 2018

Sr. No	Parameters	Bahe	Borgaon	Pundi	Audumber	Bhilwadi	Bramnal	Digraj	Sangli
1.	Temperature (^{0}c)	29	27.3	28.6	29.3	27.8	29.3	31	29.5
2.	pH	8.7	8.65	8.2	8.26	8.0	8.16	8.25	8.61
3.	TDS(mg/l)	89	102.5	100	166	162	163	215.16	229
4.	Conductivity (µ/S).	188.3	206	207	344	325	355	432	475
5.	DO (mg/l)	10.4	10.5	10.08	11.5	10.8	12.45	12.18	14.26
6.	Ca (mg/l)	1.7	2.2	4.06	2.73	2.76	2.2	4.48	6.9
7.	Mg (mg/l)	0.7	0.4	1.7	2.4	1.38	0.53	2.31	2.8
8.	Na (mg/l)	0.51	0.51	0.15	0.94	0.91	0.51	0.77	1.3
9.	Carbonate (mg/l)	0	0	0	0	0	0	0	0
10.	Bicarbonate (mg/l)	1.05	1.2	1.9	1.36	1.25	1.13	2.08	2.2
11.	Chloride (mg/l)	0.51	0.6	1	1.11	1.43	0.73	1.9	2.7
12.	Sulphate (mg/l)	1.51	1.38	3.38	2.36	2.86	1.56	4.3	4.09

(all the parameters expressed in mg/lit expect pH and EC (mmhos).

The temperature remains 27 to 31 (^{0}c) during study period. The maximum temperature is 31 in Digraj .It is graphically represented in Fig. No. 2. Temperature is an important parameter, which is directly related with the chemical reaction in the water and biochemical reaction in the living organisms. Usually water temperature is lower than air temperature in rainy season. The pH is always alkaline. The pH were decreased in summer and increased in winter similar trend was given by Mathivanan *et al.*, 2005. The pH value found increased due to activity of green algae which consumes Co_2 dissolved in water.The total dissolved solids are 89 to 229 (mg/l). The present result stated that total dissolved solids content decreases with downstream of river except Bahe. This is due to absorption of dissolved salts on earth surface and natural purification system of river. The maximum TDS are in Sangli site (229 mg/l) as compare to other study site. Similar trends were given by Prasad and Patil (2008). In rainy season

Electrical conductivity value is decrease due to addition of rain water. The Electrical Conductive ranges 188 to 475 (µ/S). It is observed that Electrical conductivity of river water constantly goes on increase this is due to socioeconomically activities are carried out at river site. EC value depends upon dissolved solids (APHA, 2005). The level of dissolved oxygen of water may vary from place to place due to variation in the temperature. Dissolved oxygen is remains 10 to 14 (mg/l). The value of dissolved oxygen is higher in Bramnal and Sangli site. Temperature is increased in summer and dissolved level is decrease similarly temperature is decrease in winter and dissolved oxygen level increase. Tiwari and Ranga (2012), Sinha and Biswas (2011) stated that the trend of dissolved oxygen maximum in winter and minimum in summer. Similar results were obtained to Patil *et al.*,(2013) and Manjare (2014). The calcium content is low in Borgaon, Bahe and Bramnal and very high in Sangli and Audumber during study period. The value ranges 1.7 to 6.9 (mg/l). The value of calcium is low in rainy season due to more dilution of water and high in winter due to concentration of calcium ions from rock and soil. Similar result were obtained to Patil *et al.*,(2013), Venkatsubramani and Meenmbal, (2007) stated that Magnesium is often associated with in all kind of water but its concentration remains lower than the calcium.

In present study, the values of magnesium are obtained 0.4 to 2.8 (mg/l).The value of magnesium is more in Sangli and Digraj while low in Bahe. Similar result were obtained to Patil *et al.*,(2013), Manjare (2014) and Verma *et al.*,(2011). The values of sodium are lower in winter. It ranges 0.5 to 1.3 (mg/l). The minimum value of sodium (0.15 mg/l) in Pundi while maximum value (1.3 mg/l) in Sangli. Similar results were find by Garg et al., 2011. The bicarbonate are occurs 1.05 to 2.2 (mg/l). The minimum bicarbonates are in Bahe and maximum in Sangli study site. The chloride content is low in Bahe while high in Sangli. It remains 0.5 to 2.7 (mg/l). The chloride values were decreased in rainy season due less anthropogenic activities (Garg *et al.*, 2011). The chief source of sulphate is from agriculture runoff. The sulphate value ranges from 1.38 to 4.3 (mg/l). The value of sulphate is low in Borgaon while high in Digraj this due to maximum mineral and organic sulfur run from catchment area (Latha and Mohan 2010).

4. CONCLUSION

In present study conclude that the studied physico-chemical parameters are within the permissible limit as WHO, ICMR, EPA and Bureau of Indian standard norms for drinking (Table No. 2). Therefore studied area of Krishna river, water is not much harmful and use for drinking and other economic sources such as fish culture.

Table2. Comparison of water parameters with Bureau of Indian standard, WHO, ICMR and EPA values

Sr. no.	Parameter	Technique used	Bureau of Indian standard (BIS)2009	WHO standard 2011	EPA guidelines	ICMR Values
1	TEMP(0^{0}C)	Thermometer	-	-	-	-
2	CONDUCTIVITY US/CM	Conductivity meter / Water analysis kit - -	750	750	2500 us/cm	570-4900
3	DISSOLVED OXYGEN(PPM)	Redox titration	5	-	-	4-6 ppm
4	PH	pH meter	6.5-8.5	7.0-8.5	6.5 – 9.5	6 - 8.5
5	TDS	TDS meter	500	600	-	500 -1500
6	TH	Complexometric titration	200 ppm	300 ppm	< 200 ppm	300
7	CALCIUM	Complexometric titration	75	100	-	75
8	MANGNECIUM	Complexometric titration	150 ppm	30 ppm	-	50
9	SODIUM	Flame Photometer	200 ppm	180 ppm	200 ppm	45-100 ppm

10	CARBONATE	Titration	-	-	-	-
11	BICARBONATE	Titration	- -	-	-	-
12	CHLORIDES	Argentometric titration	250 ppm	250 ppm	250 ppm	1000
13	SULPHATE	Nephelometer / Turbidimeter	200 ppm	200 ppm	250 ppm	400

Ref.:- [WHO, USEPA, Indian Standard, National Primary Drinking Water Regulations, Drinking Water Contaminants US EPA]

Fig. No.2 Average temperature (Oc) of study site

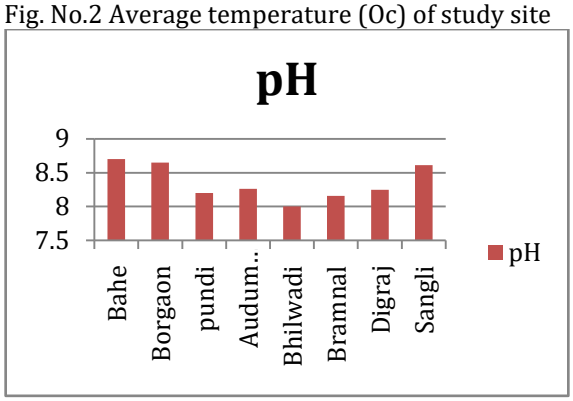


Fig.No.3 Average pH of study site

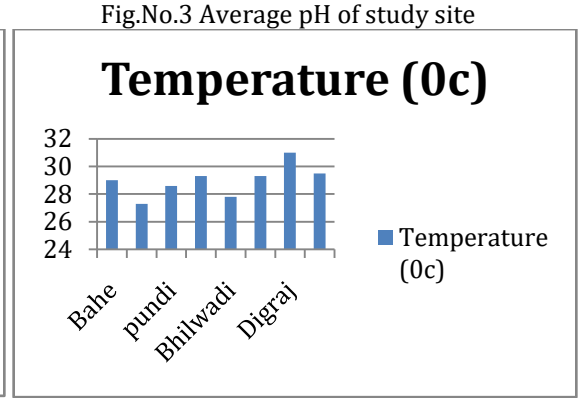


Fig. No.4 Average TDS (mg/l) of study site

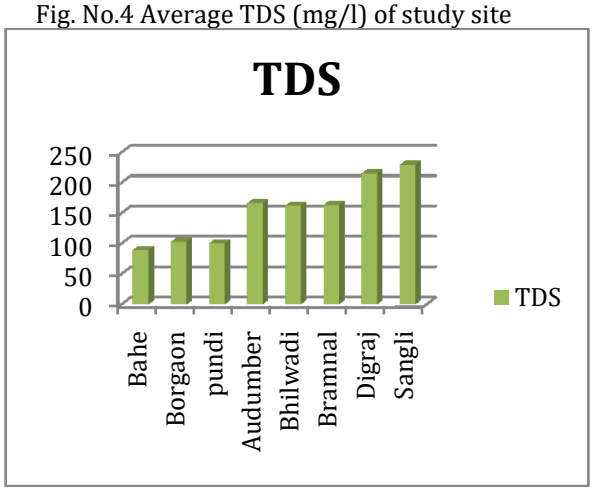


Fig. No.5 Average EC (**µ/S)**of study site

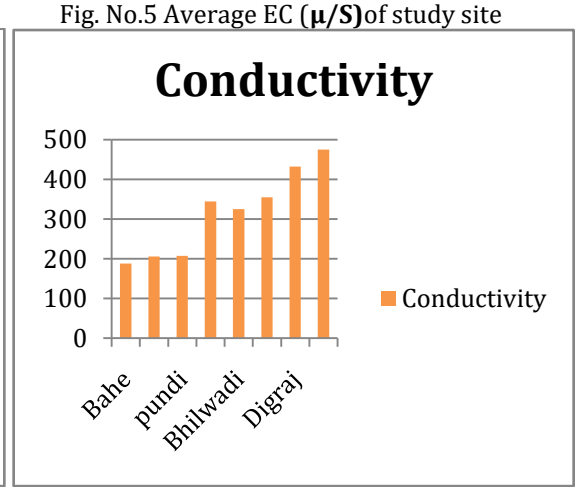


Fig. No.6 Average DO(mg/l) of study site

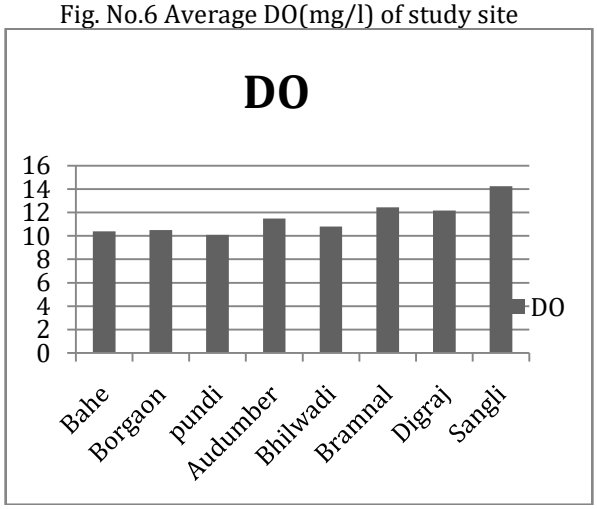


Fig. No.7 Average Ca (mg/l)of study site

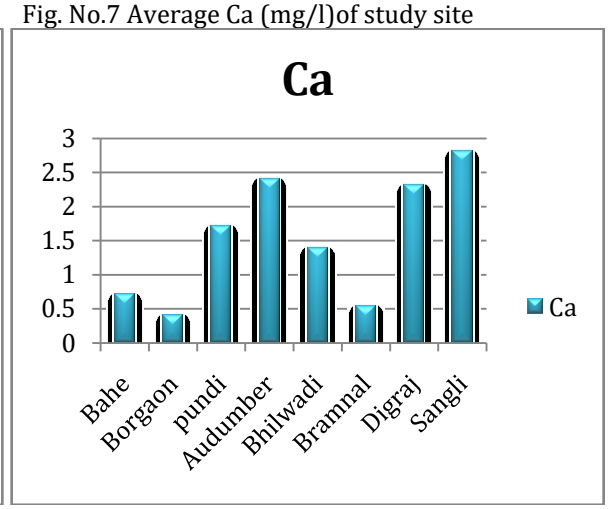


Fig. No.8 Average Mg (mg/l) of study site

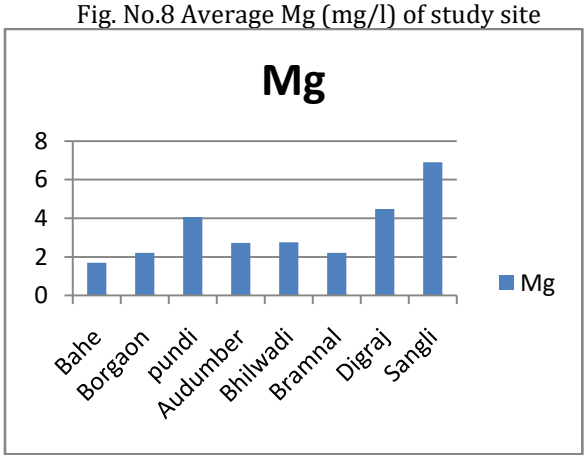


Fig. No.9 Average Na (mg/l)of study site

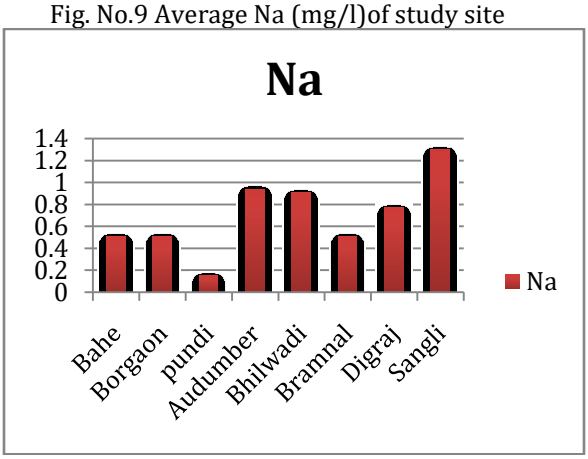


Fig. No.10 Average Bicarbonate (mg/l)of study site

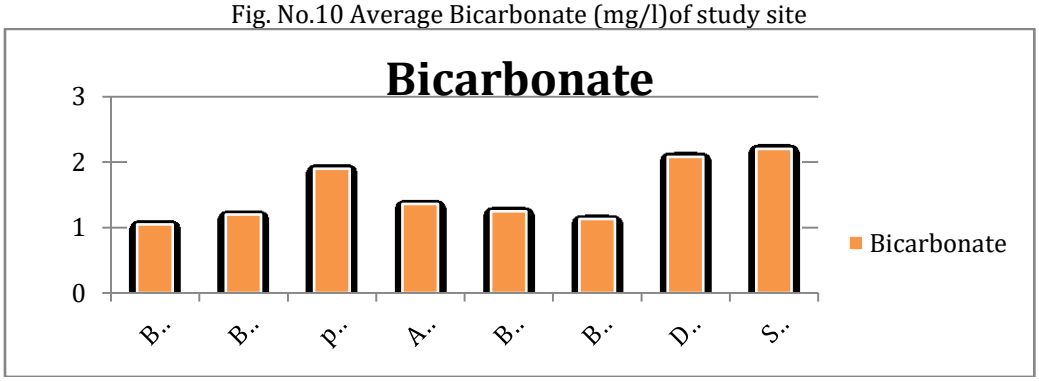


Fig. No.11 Average Sulphate (mg/l)of study site

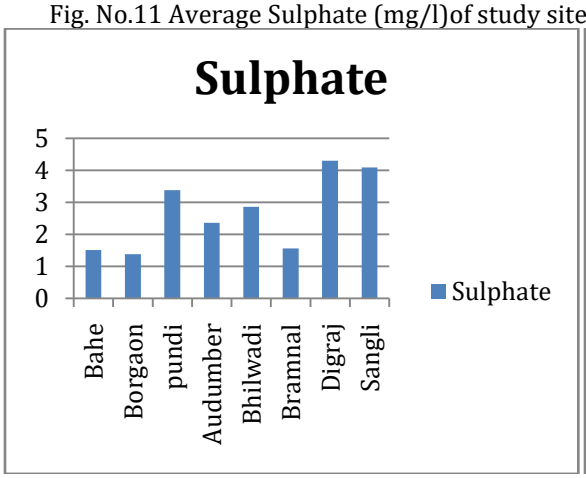
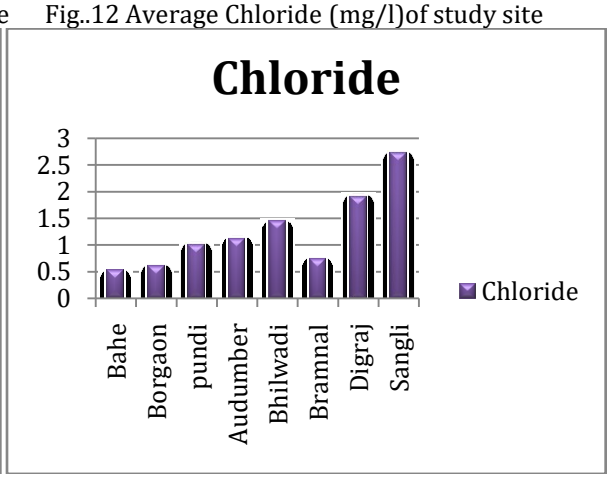


Fig..12 Average Chloride (mg/l)of study site



5. ACKNOWLEDGEMENTS

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STUDIES ON LIPOLYTIC ACTIVITY IN HAEMOLYMPH AND FAT BODY DURING LARVAL GROWTH OF LEMON BUTTERFLY, *PAPILIO DEMOLEUS* (LINNAEUS)

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ABSTRACT: *Studies on lipolytic activity in haemolymph and fat body during larval growth of lemon butterfly, Papilio demoleus (L.) have been attempted. The lipase activity revealed 0.298 and 0.379 µ-Equ. FFA/100 mg/25 minutes in haemolymph and fat body respectively during larval growth of P. demoleus. One-way analysis of variance (ANOVA) of lipolytic activity from 6 to 12- day larvae of P. demoleus was found to be p<0.027. The significance of lipase during larval growth of P. demoelus has been discussed.*

Keywords: *Lipase (EC 3.1.1.3), larval haemolymph, fat body, P. demoleus.*

I. INTRODUCTION

The lemon butterfly, *P. demoleus* belongs to order-Lepidoptera of class Insecta and it is destructive pest of citrus in India (Tembhare, 1997). Insects are a highly specialized group of invertebrates belonging to the largest of animal phyla, the Arthropoda. It is not an exaggeration to say that practically three-fourths of the animal species come under this phylum. Insects are very successful animals. Of the estimated 1.35 million living species of animals more than 900,000 are insects. Insects are abundant in places where green vegetation is luxuriant, but they are also found in myriads in dead and decaying organic matter. They also exist as parasites on and in animals including other insects. Man has been attracted by insects from very early times. In ancient India, philosophical commentaries and literature mention about insects. The Sanskrit term shatpada refers to the hexapodous condition of insects (David and Ananthakrishnan, 2006).

A few studies have been carried out in *P. demoleus* which is pest of citrus (Eastwood *et al*., 2006 and Lewis *et al*., 2011). The provenance of swallowtail butterflies, *P. demoleus* recently discovered in the New World (Eastwood *et al*., 2006). Lewis *et al*. (2011) have been reported efficacy of methionine against *P. demoleus.*

Many attempts have been made to investigate triacylglycerol ester hydrolase activity in insect species (Chattopadhyay, 2011; Khosravi *et al*., 2011 and Santana *et al.,* 2017). The information on lipolytic acivity in haemolymph and fat body during larval growth of lemon butterfly, *P. demoleus*is rather scanty.

In the present work, an attempt has been made to estimate lipolytic activity in haemolymph and fat body during larval growth of lemon butterfly, P. demoleuswhich is mainly concerned with release of free fatty acids for energy and structural components of larva.

II. MATERIALS AND METHODS

The culture of *P. demoleus*was maintained on the leaves. The larvae from 6 to 14-day were selected for triacylglycerol ester hydrolase activity. For the enzyme preparation larval haemolymph and fat body were isolated under ice cold distilled water, weighed and homogenized in the cold distilled water using a ground glass mortar and pestle. The homogenate were diluted with cold distilled water so as to get 1 % (wt/vol) concentration. Such homogenate were used for the assay of triacylglycerol ester hydrolase (EC 3.1.1.3) activity (Pol and Sawant (1997). The assay system contained 0.25 ml of 6 % olive oil dispersed in gum acacia; 1.0 ml of 0.2 M tris-maleate buffer pH 7.7 for fat body and pH 7.4 for haemolymph, 0.25 ml of 1 % (wt/vol) enzyme solution in a total volume of 1.5 ml. The incubations were carried out in a shaker with a continuous shaking for 25 minutes in glass stoppered vessels at 37 ^{0} C. The reaction was stopped with 2 ml of Cu-TEA reagent (1N acetic acid :1M 2,2',2" trinitrilloethanol:6.45% $Cu(NO_3)_2$,(1:9:10,v/v/v/). The colour was developed by the addition of 1 ml of 0.5 % solution of mixture of diphenyl carbazone

and diphenylcarbazid (5:95 w/w) in methanol. At the end of the incubation the liberated fatty acids were measured colorimetrically according to Itaya (1977).

III. RESULTS AND DISCUSSION

RESULTS:

Studies on lipolytic activity in haemolymph and fat body during larval growth of lemon butterfly, P. demoleus have been attempted. The maximum lipase activity revealed 0.298 and 0.379 µ-Equ. FFA/100 mg/25 minutes in haemolymph and fat body respectively during 9-day larval growth of P. demoleus. One-way analysis of variance (ANOVA) of lipolytic activity from 6 to 12- day larvae of P. demoleus was found to be $p<0.027$. Larval developmental period of P. demoleus is of 14-days. Lipolytic activity in haemolymph and fat body during larval growth of lemon butterfly, P. demoleus is shown in figure 1.

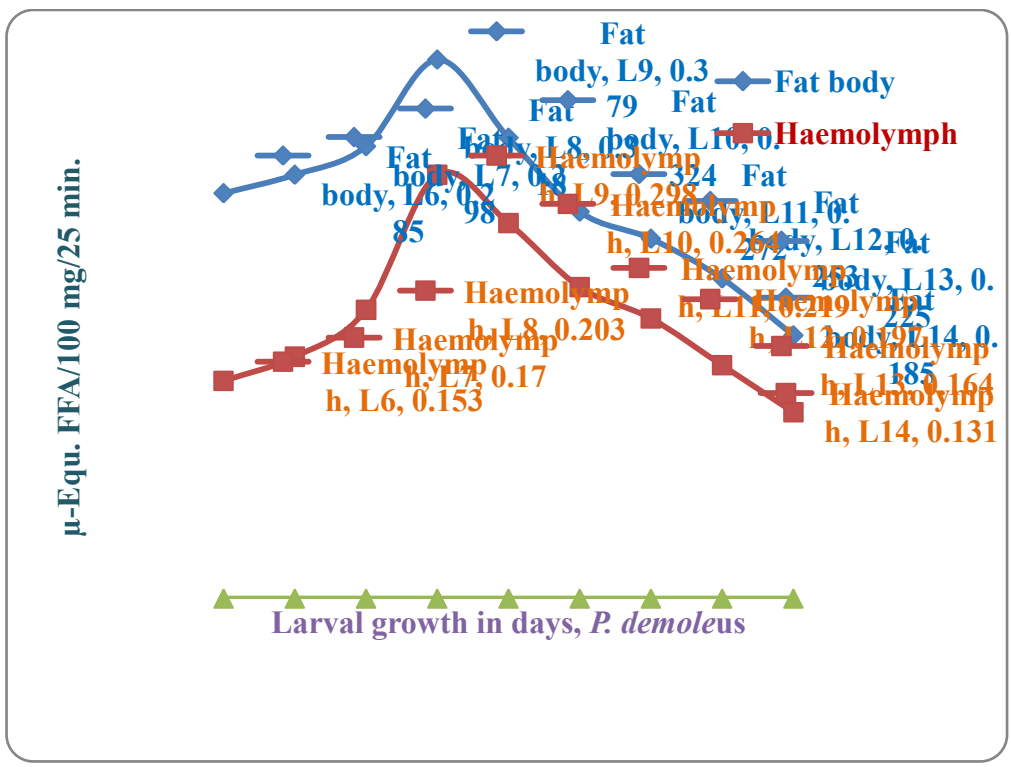

Fig.1: Lipolytic activity in haemolymph and fat body during larval growth of lemon butterfly, *P. demoleus.*

DISCUSSION:

The lipase activity in the hemolymph of tobacco hornworm, *Manduca sexta* has been reported by Chang and Friedman (1971). The lipase activity was maximal at the broad pH range 8.5 to 9.0, 1 % enzyme, 10 minutes of incubation time and increase in activity from 3-day larval fat body to 4-day larval fat body of *Chrysomyia rufifacies* Pol and Sawant (1997). Zibaee and Fazeli-Dinan (2012) have been showed lipase activity of third instar larvae of *Naranga aenescens* 4.33-fold and 2-fold more than that of the first and the second instars, respectively. Santana *et al.* (2017) have been noted lipase activity in midgut homogenate of *Rhynchophorus palmarum*larvae at 40 minutes of incubation, maximum activity at 37 °C and at pH 6.5.

In the present study, triacylglycerol ester hydrolase at pH 7.7 in larval fat body and at pH 7.4 in larval haemolymph of *P. demoleus* indicates maximum activity at an alkaline pH and reflects the microsomal lipase activity. The Michaelis-Menten constant K_m value 0.162×10^{-2} mM in larval fat body and 0.234×10^{-2} mM in larval haemolymph indicates high affinity of triacylglycerol ester hydrolase to the olive oil emulsion. The findings noted herein indicates that extra digestive alkaline triacylglycerol ester hydrolase exist in the larval haemolymph and fat body of *P. demoleus*. The gradual increase in triacylglycerol ester hydrolase activity in larval haemolymph and fat body from 6 to 9 day larvae of *P. demoleus* suggestsenergy required

for their active life may be provided by triglyceride catabolism. The decrease from 9 to 14 day larval fat body and haemolymph suggest the decreased rate of hydrolysis of triglycerides. The maximum triacylglycerol ester hydrolase activity in 9-day larvae tissues of *P. demoleus*indicates the possible mobilization of lipid in most active feeding larval stage required more energy and structural components for larval growth. The minimum triacylglycerol ester hydrolase activity of 6-day as compared to 9-day suggest histogenesis and early developmental period of larval development. The rate of release of free fatty acids in 9-day larvae is more in larval haemolymph. One-way analysis of variance (ANOVA) of triacylglycerol ester hydrolase activity in haemolymph and fat body tissues from 6-day larvae to 12- day larvae of *P. demoleus* $p<0.027$ indicates independent samples and different mean values among the haemolymph and fat body of *P. demoleus*. This finding is consisting with the fact that larvae eat constantly and accumulate lipid reserves for utilization during the adult stage. Similar findings were noted by above authors.

IV. ACKNOWLEDGEMENT:

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PRESENT STATUS OF FISH CONSUMPTION IN SATARA CITY

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ABSTRACT: *The survey was conducted during the period of 2016-Nov 2018 at various regions of Satara city through personal interviews to access region specific consumption rates by targeting the residence of the respondent. The relevant questions were asked to know knowledge of the respondents of a particular population. The purpose of the survey was to study consumer attitude towards fish consumption that can be currently determined by parameters such as price, taste, quality, availability or hygiene at point of sale. The survey resulted in some interesting observations. In order to satisfy increasing demands many marketing strategies could be evolved and, simultaneously, help could be offered for the development and management of fisheries.*

Keywords: *Respondent, consumption, consumers, productivity, key influence*

I. INTRODUCTION

A fish market is a marketplace for selling fishproducts. It can be dedicated to wholesale trade between fishermen and fish merchants, or to the sale of seafood to individual consumers, or to both. Retail fish markets, a type of wetmarket, often sell street food as well.Because seafood is quick to spoil, fish markets are historically most often found in seaside towns. Once ice or other simple cooling methods became available, some were also established in large inland cities that had good trade routes to the coast. Since refrigeration and rapid transport became available in the 19th and 20th century, fish markets can technically be established at any place. However, because modern trade logisticsin general has shifted away from marketplaces and towards retail outlets, such as supermarkets, most seafood worldwide is now sold to consumers through these venues, like most other foodstuffs.

Fish are a great choice for serving up lean protein with plenty of vitamins and minerals. Fish also are a natural source of omega-3 fatty acids - a good kind of fat! The omega-3 fatty acids found in fish are called EPA and DHA. Our bodies cannot make EPA and DHA. Eating fish is the main way to get these important fatty acids that you do not get from other foods. (Supplements may not be as beneficial.) Here is the best part:DHA is a building block of the brain and eyes. Pregnant women and breastfeeding moms can eat fish to give DHA to their babies. Eating fish can lower the risk of heart disease

The survey of market for fish consumption preferences have been carried out by many researchers Khan and Jafery 1993, Welch 2007,Jamdade et al 2011, **Yaqin Hu et al 2014 ,** Mehmet Ferit Can et.al 2015,Aishat T Bakre et al.2016 Ravi Shankar Kumar 2017 and Atin Supartini et al ,2018. They studied the fish consumption preferences on the basis of socio-economic status, taste, pollution free food, and changes in preferences across the years. The different aspects if studied form an important guideline for marketing of fishes. It also brings forth important observations related to the price paid by fish consumers for taste irrespective of the nutritious value. To find such traits in the local markets of Satara and adjoining areas of the city the present study was carried out.

II. MATERIALS AND METHODS

There are various methods described for conducting consumption surveys like telephonic surveys, online surveys, personal interviews, group discussions etc. Of these four categories, telephonic interview method was applied. In thismethod questions were asked to know knowledge of therespondents of a particular population. Pollock 1994 suggested creating a data requirement by asking questions for the questionnaire to confirm that each question is relevant to study the objectives. There for questions asked to the respondents were in regional language that specifies all information and requirements necessary to adequately describe the consumption patterns for the target population.

Study Area

The fish vendors and the places of fish sale were identified after careful study. All the markets, stalls and places where fish are sold either daily or during weekly markets were selected. The survey was conducted in 2016-Nov 2018 at various regionsof Satara city such as Satara old motor stand fish market, Sadar bazar, Bombay restaurant, Godoli, Deshmukh colony, Devi colony, Ravivar peth. Also the shops far from fish

market such as DaryaDaulatMasali Centre,Parvej Fish Center, Marium Fish Center,Ishan Fish Center, Garde Fish Mall, Patil Fish Stall.

Target Respondent

The target respondents for this study were in two categories: a) Housewife. b) An additional member of the family.

Sample Size and Selection

A total of 1000 interviews were conducted across the entire city including all categories of respondents. Against this, a totalof 1000interviews were received. The starting points were selected in Satara, which were nearer to the fish market (Satara old motor stand). Around each starting point about seven to eight families were selected using the Right Hand Rule, that is, byusing random walk method which eliminates interviewer bias in selection of a family. Therespondent was then interviewed to determine the incidence ofindividual's fish consumption, if any, in such households.

Analysis

Analysis of fish consumption was done by location and category of household. Throughout the report an attempt has been made to include data which clearly demonstrates significant trends and differences.

RESULTS

1) Distribution of families according to their diet:

Table 1: Distribution of families according to their diet

Family Members	1 – 4	5 – 8	9 – 12	13 – 16	16 - 20	Total
Total Members	400	320	180	50	50	1000
Vegetarian	108	67	57	12	10	254
Both (Non-veg)	292	253	123	38	40	746
% of Vegetarian	27%	20.9%	31.6%	24%	20%	25.4%
% Of Both (Non-veg)	73%	25.3%	12.3%	76%	80%	74.6%

From the table it is clear that number of non-vegetarians in the families considering 1 to 8 members were highest of about 75.69% than in large families considering 12 and more than 12 members of about 71.78%. The moderate families considering 9 to 12 members showed less consumption of non-vegetarian food of 12.3%. The diet preference is based on both, personal choice as well as number of individuals in a family.

2) Different types of fish foods according to their consumption:

Table 2: Different types of fish according to their consumption

Types of Fish	Marine	Fresh water
Fish	210	237
Crabs	80	65
Prawns	69	78
Lobsters	44	33
Others	27	20

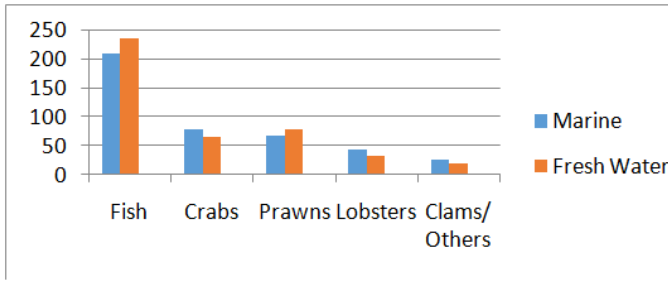


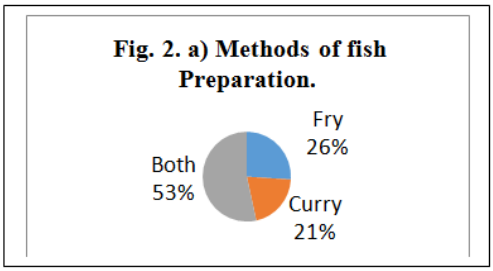
Fig.1. Types of fish food according to their consumption

Among the fish consumers interesting data was obtained regarding preference between fresh water and marine waterfish (Fig.1). In case of fin fish most of the individuals' preferred marine fish than fresh water fish. Fresh water crabs outnumbered marine. In case of prawns, lobster and calms they were mostly preferred against fresh water.

3) Methods of fish preparation and knowledge about fish byproducts:

a) Table 3: Methods of fish Preparation

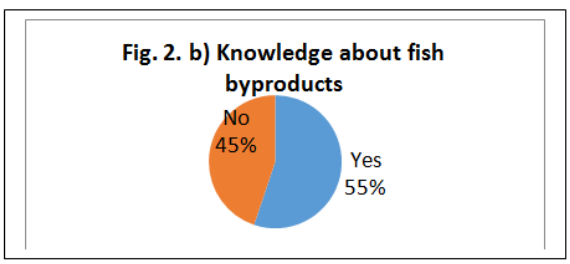
Methods of fish Preparation	No. Of Families	Percentage
Fry	130	26%
Curry	103	20.6%
Both	267	53.4%



About fish preparation (Fig. 2.a) there was seen a clear demarcation like fried gravy or both. Among fish consumers about 26% population prefers fish in as dry dish, 26.6%prefers fish as form of gravy while largest part of population (53.4%) like fish in both fry and gravy form.

b) Table 4: Knowledge about fish byproducts.

Response	No. Of Families	Percentage
Yes	276	55.2%
No	224	44.8%



The alarming condition was observed in total population regarding knowledge regarding Fish Byproducts (Fig. 3.b). Data reveals that only 55.2 % of the population is aware about fishbyproducts and their nutritional importance.

4) Distance between the nearest fish market and its Hygienic conditions:

Table 5: Distance between the nearest fish market.

Distance	No. of Families	Percentage
Less than 5 km	380	76%
More than 5 km	120	24%

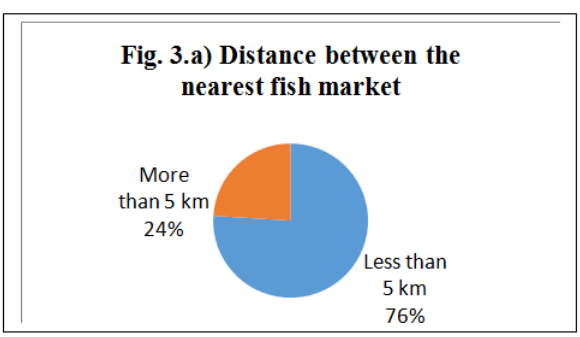
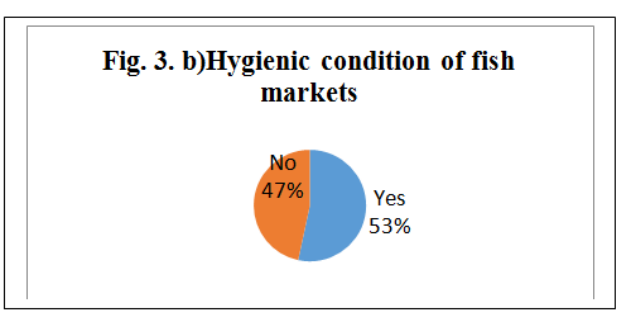


Table 6: Hygienic condition of fish markets.

Response	No. Of Families	Percentage
Yes	267	53.4%
No	233	46.6%



Individuals were asked for the distance between their house and nearest fish market (Fig.3a), interestingly 76%population is having a fish market in periphery of less than5km. While about 24% population has to go more than 5km topurchase fish so they prefer to purchase from vendors nearby.The population living near fish market reveled thatconsumption pattern is not supporting consumption rate.Consumers are happy with the hygienic conditions ingovernment fish market (Fig 3b). While some of

the populationis unsatisfied about cleanliness, drainage system.

5) Awareness about nutrition among the families depending on education of housewives'.

Table 7. Distribution of families having awareness about nutrition depending on education of housewives'.

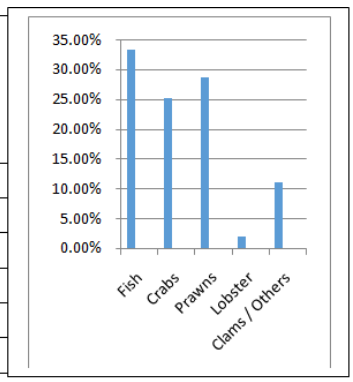
Awareness about Nutrition	Non-Educated	Primary Educated	Well Educated	Percentage
Yes	13	121	107	48.2%
No	54	119	83	51.2%
Total	67	240	193	

There were satisfactory observations in all the three categories (Table 7). It was seen highest in well-educated 55.44% followed by primary educated and non-educated withvalues 50.4% and 19.4% respectively. Data clearly showsdirect relationship of education with knowledge about nutrition. In rest of the unaware population fish is consumed first for a change in diet and especially due to delicious taste of fish flesh.

6) Frequency of consumption

Table 8.Frequency of consumption

Food Particul-ars	Daily	Weekly 2or3 times	Once a week	Once a fortni-ght	Once a month	Occasi-onal	Total	%
Fish	0	5	19	27	43	72	166	33.2%
Crabs	0	4	15	21	27	59	126	25.2%
Prawns	0	3	8	25	38	69	143	28.6%
Lobsters	0	0	0	0	0	10	10	2%
Others	0	0	0	2	18	35	55	11%
%	0	2.4%	8.4%	15%	25.2%	46%	500	

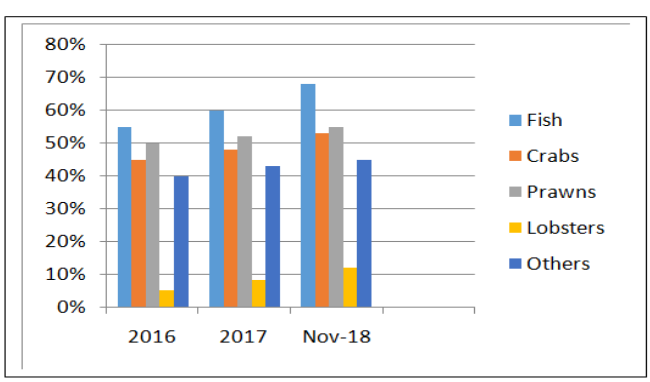


The above graph shows frequency of consumption was found to be more (11-20%) once a month while 46% were consumed on an occasional basis. It has been observed that a fish was consumed by 33.2% of the respondents while Prawns were consumed by 28.6%of the respondents. As well as crabs, Lobster and others were consumed on an occasional basis.

7)Preference of Seafood

Table 9. Preference of Seafood

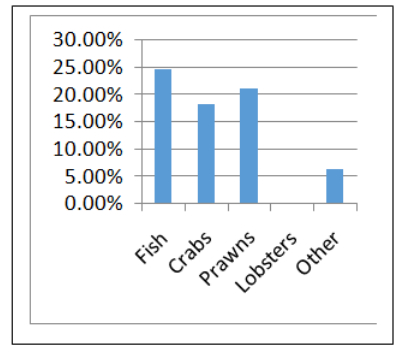
Food Particulars	Preference of fish (%)		
	2016	2017	Nov 2018
Fish	55%	60%	68%
Crabs	45%	48%	53%
Prawns	50%	52%	55%
Lobsters	5%	8%	12%
Others	40%	43%	45%
Total %	39%	42.2	46.6%



There were observations in all the three years (Table 9). It was seen highest in Nov.2018 i.e46.6% followed by 2016 and 2017 with values of 39% and 42.2% respectively. The above graph shows fish was most preferred live food as well as prawns and crabs.Different types of fish are easily available in markets and have delicious taste of fish flesh.

8) Knowledge about nutritive value of seafood

Food Particulars	Knowledge about nutritive value				Total %
	Fat	Calories	Cholesterol	Iron	
Fish	49.1%	8.9%	26.3%	36%	24.6%
Crabs	37%	6%	23.2%	24.8%	18.2%
Prawns	44.5%	6.5%	25.4%	29%	21.08%
Lobsters	0	1%	0	0	0.2%
Other	9%	2.3%	15%	6%	6.4%



Seafood is a rich source of protein, vitamins, and minerals; many varieties of seafood are also low in sodium and cholesterol. Not only is seafood delicious, but it's nutritious as well. It's a delightful addition to any meal and is an excellent, low-calorie source of many essential nutrients. Data clearly shows knowledge about nutrition values related to fish is more as compare to other seafood.

III. DISCUSSION AND CONCLUSION-

Anne Watanabe and Herald Shepherd carried out Fish Consumption Survey of the Umatilla, Nez Perce, Yakama and Warm Springs under the CRITF project and showed traditional fish consumption among the individuals of the family. In the present study it is clear that the consumption of fish depends on the number of members in the family. Jamdade et al have described the dependency of fish as food on socio-economic status and taste. In the present study the results are similar to those observed by Jamdade et al. Nurul Izzah Ahmad 2016 has stated the correlation of fish consumption and ethnic groups which holds true even in the present study.

The data reveals an interesting fact regarding the preference of fish food. There is a clear indication that the marine water fish products are having more demand than the freshwater products.

The local population opts for fried products rather than cooked products which seem to be surprising as the recent health conscious diets try to avoid oily products.

Regarding the distance required to travel the locations of fish markets require majority of the population to travel at least 5 kms to buy fish. Inspite of the demand for fish the hygienic conditions seem to be questioned as almost 50 % of the consumers are not satisfied with the conditions regarding cleanliness.

The knowledge regarding the nutritional value of the product to be consumed shows increasing trend with education. This is expected as most of the other individuals consume fish for other reasons than the nutritional value. Two important aspects that have come forward in this regard are that some population seems to go for fish products as food just for change in the regular feeding habits while some opt for fish as food because of the taste. In the later two groups nutrition plays no role in selection of fish as food. Similar studies were carried out by Ryota Hosomi 2012,

The trend of frequency in consumption of fish as food shows half the fish eaters consume fish occasionally as opposed to other animal products. Only about 2.4% seem to include fish as a regular weekly commodity in their diet. This is in agreement with the observations by Mehmet Ferit Can et al 2015 who have stated that the fish consumption is far less than the recommended value. **Yaqin Hu et al 2014 have discussed the trends in fish consumption in China with different factors and their effect on amount of fish consumed as in case of the present study. Similar studies have also been carried out by Tidwell and Allen 2001, on the consumption of fish as food and contribution of Aquaculture.**

There was observed a rising trend in consumption of fish products during the period of study. The demand for fish food has increased. This may be attributed for the increase in outlets providing fish as food, increase in development of taste for fish products and availability of fishes of the coastal area in the local markets.

As expected the consumption of fish as food is more than the shell fish products which have not yet gained the popularity. The knowledge regarding the amount of fat and other ingredients of fish is more than that of other products. Jordi Guillen 2018 has discussed the global sea food footprint. There is scope for increase in consumption of fish as food especially in the inland area.

The finding of research indicates the problem of how to augment supplies of fish to an ever-growing population in theface of dwindling coastal stocks or from inland source. Somepotential solutions suggested by the study may be:

a. Great care would need to be taken to ensure that any improvements were cost-effective to the beneficiary

while, at the same time, maintaining the affordability of the product. This is especially important with the lower income groups.

b. To assess the potential of fresh and brackish water aquaculture to provide greater quantities of fish for local markets.

c. To promote awareness of the positive health aspects of fish consumption amongst the poorest socioeconomic groups and dispel some of the myths and taboos about fish consumption.

The question arises as to who should implement theseimprovements and promotional strategies based on ongoingmarket research. Chicken, eggs, milk and several other protein food not only have more centralized and organized productionand distribution systems, but also have their own promotionalorganizations such as the Egg Produce Association of India,the Poultry Producers' Association, the Milk Marketing Boardetc. However, no such centralized body exists to support thedomestic fish marketing sector, which comprises a largenumber of unorganized small-scale operators. Such a bodycould indeed play a major future role in improving fishmarketing in India, just as similar organizations have alreadydone so, and are now doing, in other countries. Its strategy, however, would need to be highly sensitive to the diverseconsumer needs. Some of the technical interventions aimed at quality improvement, which are commonplace in other countries, simply may not be financially viable options in India.

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STUDY OF GRAPE MEALYBUG (*Maconellicoccus hirsutus)* WITH REFERENCE TO ITS NATURAL ENEMY

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ABSTRACT: ***Maconellicoccus hirsutus*** *(Green) (hemiptera: Pseudococcidae) is an invasive pest of agricultural crops. M.hirsutus causes serious losses to crop from all major grape growing regions of India (Majumnath 1985, Mani et. al.1987). Incidence of mealybug population, parasitoid, and predators was observed throughout season. Eggs, nymph, crawlers, adults of mealybugs was observed on shoot migrated seasonally towards leaves and fruits. The total life cycle of geape mealybug was shorter during summer than winter (katke and Balikai 2009). However, predators were absent during fruiting season, i e. 50th to 12th meterological standard week (MSW) (Prasanna, Balikai, Vankatesh 2015). For the study grape producing major talukas of Sangli district like Walwa, Palus, Tasgoan, Kavathemahankal and Miraj are selected. Observations were recorded on 10 plants selected at random in each vineyard on 10randomly selected shoots during the vegetative phase. This research paper attempts study of the life cycle of grape mealybug are reared on sprouted potatoes. The research of natural enemy of grape mealybug is at preliminary level. For biological control developing economically and ecologically management against pest is necessary to observe life cycle and natural enemy on grape.*

Keywords: *Grape Mealybug, life cycle, Natural enemy, Parasitoid, Predator*

I. INTRODUCTION

Grape (*vitis venifera*) is temperate fruit crop in tropical and subtropical agroclimatic condition prevailing in the Indian sub-continents.Grape is delicious, refreshing and nourishing, digestible fruit. Ripe grape is good source of minerals, vitamin B1 and B2 used for making jelly, syrup and raisin. Balikai and Kotikai 2003 recorded about 26 species of pest infesting grapevines in northern Karnataka. Out of these, grape mealybug *Maconellicoccus hirsutus* (Green) (hemiptera: Psudococcidae) is an invasive key pest of agricultural crop geographical originated in Indomalaya and currently distributed in Australia, Africa, Asia (India), Middle east, South America, Maxico and California.*M. hirsutus* has become very severe making fruit unfit for consumption, decrease crop quality by excreting abundant honeydew, which promotes sooty molds and infesting grape bunches (Flaherty et al. 1992). It feeds on grape leaves and grape bunches during summer.

Severe mealybug outbreaks have been reported in India's, adversely affecting grape production by 90% losses in the state of Andhra Pradesh. *M. hirsutus* , *Pl. citri* , *Nipaecoccus viridis* (Newstead) and the root mealybug, *X. annandalei* , are the primary mealybug pests. Cultivars harvested in late fall suffer heavily from mealybug damage. Increasing mealybug problem may be due to frequent and indiscriminate and judicious use of insecticides, which may hampers natural enemies liable for the suppression ofmealybugs. Although the root mealybug damage root by reduces vine vigor and yield and shortens fruit-bearing canes (Rajagopal et al. 1997).

*Maconellicoccus hirsutus*is the most important of the grape mealybugs in peninsular India, with severe infestations leading to berry and shoot damage. The mealybug occurs on the vine throughout the year (Mani and Thontadarya 1987c). After harvest, the mealybug population is confined to vegetative parts, where it overwinters. In spring, the vines are given a 'foundation pruning' usually in April–May and *M. hirsutus* remains on the leaves, stem, and trunk from this period until harvest. From mid to late summer, the population density is typically decrease until late fall when the vines are given a 'berry pruning'. The mealybug population density starts increasing from mid-December onwards and by January (midwinter). *M. hirsutus* population migrates from the trunk, cordons, and shoots to developing berries. The pest population build-up coincides with high temperatures (30–40°C), low humidity (less than 40%) and berry development. Peak population is reached before harvest in spring (March–April). An early harvested crop usually reduces mealybug damage as compared to a late harvestedcrop. Also, heavy rains and cool temperatures of less than 20°C can result in a temporary reduction in the *M. hirsutus* population, often encountered in winter and rainy seasons.

For biological controls, six parasitoids and seven predators have been associated with *M . hirsutus*. The parasitoids are *Anagyrus dactylopii* (Howard), *Allotropa* sp. nr. *japonica* Ashmead, *Anagyrus mirzai*

Agarwal & Alam, *Alamella fl ava* Agarwal, *Leptopilinia* sp., and *Chartocerus* sp. nr. *walkeri* Hayat. The predators are *Scymnus gratiosus* Wiese, *Scymnus coccivora* Ayyar, *C. montrouzieri* , *Chrysopa* sp., *Spalgisepius* Westwood, *Cacoxenus perspicax* (Knab), and *Triommata coccidivora*.Among these, *A. dactylopii* and *S. coccivora* are the most important, causing up to 70% parasitism (Mani et al. 1987).

II. MATERIAL AND METHODS

Well established grape gardens located at the sangli district will be selected for studying the seasonal incidence and life cycle of mealybug, *Maconellicoccus hirsutus.*For the study grape producing major talukas of Sangli district like Walwa, Palus, Tasgoan, Kavathemahankal and Miraj are selected. Observation were recorded on 10 plants selected at random in each vineyards and 10 randomly selected shoots during vegetative phase This research paper attempts study of the life cycle of grape mealybug are reared on sprouted potatoes.

1. **Life Cycle of *Maconellicoccushirsutus:***

Eggs: Eggs appeared yellow to orange red, 0.3 to 0.4mm length laid in groups 150-600 in terminal ovisacs in period of weeks. Eggs hatch in period of 6 to 9 days within an eggs sacs. Longtailed mealybugs give birth to live crawlers.Vinemealy bug has high reproductive rate, with some female depositing more than 250 eggs, and fast developmental time with 4to7 generation per year.

Crawlers: All species are yellow to orange-brown or pink colour, 0.3mm long, highly mobile easily carrier of by winds. The first instar referred as crawlers. They move and settle over plant parts to initiates feeding action. There are three nymphal instars in female and four in males which lasts for 22-25 days. The third instar nymph of male forms cottony white cocoon inside which the population occurs. All the nymphal stages of the female looks similar except for the difference in size. The total nymphal period is 21 days for male and 19 days for female mealybugs. The last instar of male is inactive stage with winged bud and within cocoons of mealy wax. The grape mealybug has two generations each year and overwinters as an egg or crawler in or near a white, cottony egg sac hidden under loose bark and in the cordons or upper portions of the trunk. In spring, crawlers move toward the base of spurs or under the loose bark of canes and then onto expanding green shoots reaching maturity in mid-May to early June. Most females return to old wood to lay eggs that hatch from mid-June to July. First generation crawlers then move out to the green part of the vine to feed on fruit and foliage in late June or early July. Mostly immature females are observed throughout July. Immature females and newly matured females are grayish pink colour.

Image 1; Infestation of mealybug on stem

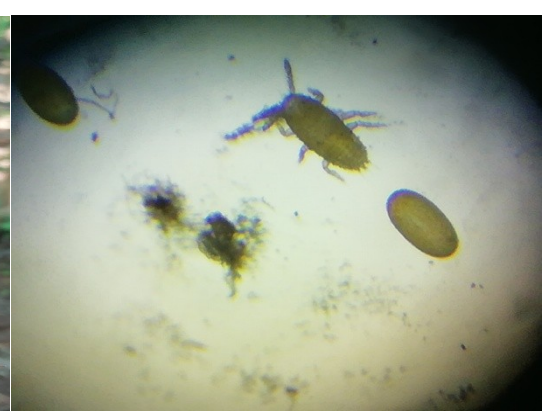

Image 2; Egg and Crawlers stageof mealybug

Adults: Adult female mealybug is small to pinkish or yellowish white, 2.5 to 4mm long soft bodied, elongated oval slightly flattened body covered with white waxy secretion or filament sticking out body. Longer filaments from the posterior end make these mealybugs appear to have "tails."Females show the combination of 9-segmentedantennae, anal lobe bars, numerous dorsal oral rim ducts on allparts of the body except the limbs and long, flagellate dorsalsetae. It will appear in late summer and early fall. Some females will oviposit in the fruit clusters but the majority of the females return to the old wood to lay the overwintering eggs. Grape mealybug overwinters under the bark of the trunk, cordons, and spurs. In late spring, some mealybugs begin to feed on leaves, but the majority of the population remains hidden under the bark or in the tight clusters.

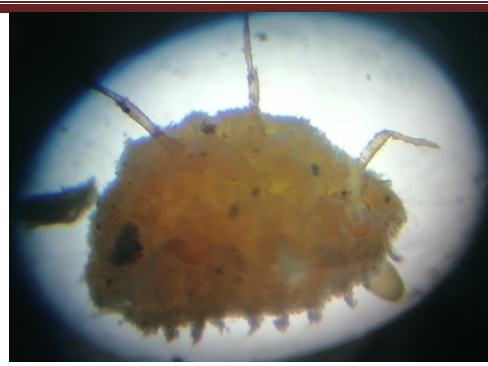

Image3; Nymphal stage of mealybug

Image4; Adult of mealybug

The grape mealybug and the obscure mealybug closely resemble. If the color of the fluid excreted is reddish orange, then it is most likely grape mealybug. Another distinguishing characteristic is based on the different life cycles of the two species: grape mealybug diapauses in winter and has two generations a year that do not overlap. If only one or two life stages of a mealybug are present at a given time, it is most likely grape mealybug diapauses.

Male mealybugs are rare and female occurs throughout the year. Males have one pair of very simple wings, long antennae, whitewax filaments projecting posteriorly and no mouthparts. They are capable of reproducing parthenogenetically but some biparental. Life cycle completed within 30 days under favourable condition. Mealybug difficult to eradicate they have protective mealy covering on body. It forms colonies in protected areas like cracks and crevices. Both nymphs and adults cause damage by sucking sap by piercing and sucking from stem, cordons, buds aerial roots, shoots, flower panicles and bunches. Infestations of *M. hirsutus* are often associated with attract ants (Ghose, 1970; Mani, 1989). Mealybug infestation on bunches reduces sugar content in juice which in turn reduce grape quality. When feeding on stem, they remain concealed below loose bark thus get hardly exposed to chemical spray. Feeding on crop by mealybug can be severe for stunted growth and defoliation. During February, March and April mealybug development attains faster space due to high temperature and abundant availability of food. Once sugar development starts in berries, it is difficult to eradicate mealybug population.

III. Prevention:

Cultural, mechanical, biological, and chemical methods of control have to be integrated to reduce the mealybug populations and reduce berry damage. After pruning mealybug infestation always start in scattered patches during December to January. Spraying methomyl 40 SP before flowering is recommended.Release exotic predator, *Cryptolaemus montrouzieri* 10 beetles per vine for biological method of control.

IV. CONCLUSION:

The present study report suggest study of life cycle and seasonal changes in mealybugs eggs, crawlers, nymphal stages and adults and its infestation with reference to natural enemies to eradicate as biological control to reduce mealybug population.The predictions of potential distribution of the pests are helpful in developing strategies for monitoring and managing these serious pests.

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STUDIES ON EFFECT OF NICKEL ON PROTEIN CONTENT OF KIDNEY OF FRESHWATER FISH – *ETROPLUS SURADENSIS*

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ABSTRACT: *The work was planned to investigate the toxic effect of Nickel Nitrate on biochemical composition of Etroplus suradensis. Preliminary studies were conducted using Nickel nitrate to find out the lethal concentration LC50 for 24 hours by following the method of Finney (1971). LC50 for 24 hours was found to be 0.05%. Fish were then exposed to sub lethal concentration of Nickel Nitrate for 24 hrs, 72 hrs and 96 hours; fish from control and experimental medium were dissected out and kidney was gently separated and preserved in deep fridge keeping them in plastic containers. Extract of tissues was made in distilled water by using homogenizer. Protein was determined by Lowry's method (1951). The protein content in kidney of the animals treated with lower concentration dropped down significantly with increase in period of exposure.*

I. INTRODUCTION

Metals are commonly found in the environment, they are present as a natural element or as a result of anthropogenic activities in different environmental media, such as air, water and soil, which constitute an important factor of exposure to animals and human, (Louis, 1993). Heavy metals are considered as one of the most important factors which affect fish population, reducing their growth, reproduction and / or survival rate. (Mohamed and Saleh, 1996). Nickel is one of the microelements which occur in trace amounts in living organisms. It constitutes a potential hazard to the environment media (air, water and soil). This is due to its extensive and wide spread utilization in various industries; it is a common by product of electroplating industries, steel production, metal mining, smelting, refining, ceramic and processing along with fuel combustion and waste incineration activities. Effluents that spread to streams, rivers and lakes may disrupt the integrity of the aquatic environment. Excess of nickel contamination is a real hazard to aquatic ecosystems due to its persistence and bioaccumulation. (Atchinson*et. al*., 1987).

Environmental exposure of nickel occurs through inhalation, ingestion and dermal contact. The general population is exposed to high levels of nickel because it is widely present in air, water, food and consumer products. The general population is exposed to nickel in nickel alloys and nickel plated materials such as coins, steel and jewellary and residual nickel may be found in soaps, fats and oils. (ATSDR, 1997). It induces decrease in body weight of *Oreochromis niloticus* fish. (El- Saieed and Mekawy, 2001). Little studies have investigated nickel uptake in fish through aqueous and dietary exposure. Further research investigating the exposure of fish to dietary nickel, which is needed to elucidate the potential impact of chronic dietary nickel exposure on natural populations of fresh water fish.(Ptashynski and Klaverkamp, (2002).

In order to investigate the variations in relation to biochemical components such as protein have to be estimated in kidney of the fish as protein shows fluctuation.

The biochemical changes occurring in the body gives first indication of stress. Organisms require sufficient energy during stress. The energy is supplied from reserve food materials like Glycogen, Protein and Lipid. When stress is mild, only stored glycogen is used as a source of energy, but if stress is strong the energy stored in protein and lipid may be used. As different tissues and organs have different activates, their metabolism is different and hence, biochemical changes due to a particular pollutant in different issue of the same organism may be different.

Etroplus suradensis fish are selected as a research fish model, because these fish are easily produced and economically important. Fish are known by their tendency to localize significant amount of metals. They absorb metals from water through gills, skin and digestive tract.

Proteins are important organic constituents in the tissue of animals, which play an important role in cellular metabolism. Several investigators reported that the heavy metals interfere with protein synthesis. Hence an attempt was made to study changes in a total protein of kidney tissue in *Etroplus suradensis* in present study.

II. MATERIALS AND METHODS:

For present study, healthy fishes (*Etroplus suradensis*) having equal size, measuring 20 cm and equal weight nearing 200 grams were collected from River Godavari from Nanded city. Fish were transported by car, keeping them in plastic container having 50 liter water capacity. Firstly fishes were acclimatized for seven days in Raceways constructed in N.E.S. Science College campus, Nanded and then in plastic tubs having 20 liters water capacity in laboratory for next seven days.

During acclimatization, fishes were fed with rice bran and ground nut oil cake in ratio of 1:1 by weight. Food was supplied daily @ 2% to the total body weight of fishes.

Preliminary studies were conducted using Nickel Nitrate to find out the lethal concentration LC 50 for 24 hours by following the method of Finney (1971).

Fish were then exposed to sub lethal concentration of Nickel Nitrate for 24, 72 and 96 hours, fish from control and experimental medium were dissected out and kidneys were gently separated and preserved in deep fridge keeping them in plastic container.

Extract of tissues was made in distilled water by using homogenizer and proteins were estimated by Lowry's method (1951). Results are presented in tabular and graphic form. Variance and standard deviation are calculated and expressed in table. The significance was tested by following Student's t – test for two means small samples.

III. RESULTS AND DISCUSSION:

The protein content in kidney of the animals treated with lower concentration dropped down significantly with increase in period of exposure. A similar trend was found in protein content of kidney when the animals were treated with higher concentration (Table 1 and Figure 1).

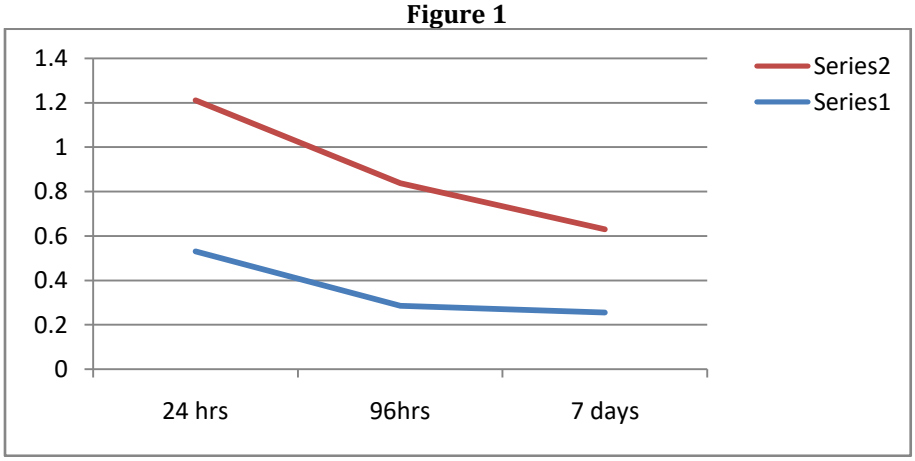
Table 1

Effect of Nickel Nitrate on protein content of kidney tissue of *Etroplus suradensis*.

Conc. Of the Medium Duration	Control	1/3 Conc. (0.017%)	2/3 Conc. (0.034%)
24 hours (LC50)	0.52 ± 0.236	0.44 ± 0.272 84.61% t= - 4.445 (s)	0.55 ± 0.167 105.76% t= 0.2076 (s)
24 hours 1 Day	0.52 ± 0.236	.053 ± 0.321 101.92% t= 0.0502 (s)	0.68 ± 0.150 130.76% t= 1.1445 (s)
96 hours 4 Days	0.52 ± 0.236	0.285 ± 0.318 54.80% t= - 1.1869 (s)	0.552 ± 0.192 106.15% t= 0.2104 (s)
7 Days	0.52 ± 0.236	0.255 ± 0.278 49.03% t= -1.4824 (s)	0.375 ± 0.193 72.11% t= - 0.9515 (s)

(s) = Significant.

Figure 1



LC50 for 24 hours was found to be 0.05%. Heavy metal Nickel cause stress to organism and change in metabolic activity. The observed biochemical changes may be due to response to nickel intoxication representing adaptive regulatory mechanism or may be due to pathological effect. The animal by changing the metabolic processes defines itself from the toxic effects as protective measure.

The present observation on total protein levels in kidney are in agreement with that of Jana and Bandopadhyaya (1987) and Jha (1991).

The initial rapid decline of protein content suggested that catabolic process was initiated by increased proteolysis to meet the demand of energy in extremely stressful condition. At later stages, the rapidity in declination became slow and gradual indicating that there was a tendency of fish to adapt to the adverse environmental stress by cellular homeostasis mechanism, which perhaps to some extent regulated the function of proteases responsible for protein breakdown of the fish.

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STUDIES ON PREPERATION OF FISH MANURE FROM FISH WASTE

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ABSTRACT: *Global production is estimated to reach 8.5billion by 2020 with parallel demand for food. Agriculture sector is facing challenges to feed this rapid burst in population by applying chemical fertilizers. Application of chemical fertilizers has robbed the soil fertility and has result in health and environmental hazards. Organic waste does not contain toxins or carcinogenic material like other chemical fertilizers and are found to improve the soil structure, water holding capacity, microbial biomass and nutrient availability. Hence the alternative way for sustainable solid waste management and agriculture.*
In present study an attempt is made to use fish waste for production of fish manure. In this study it is seen that the NPK concentration of soil was increased considerably.

Keywords: *Manure, Organic waste, NPK, Chemical Fertilizer*

I. Introduction

Global population is estimated to reach 8.5 billion by 2020 with parallel demand for food. Agriculture sector is facing the daunting challenge to feed this rapid burst in population by applying chemical fertilizers. Applications of chemical fertilizers have robbed the soil fertility and have result in health and environmental hazards. Organic waste does not contain toxins or carcinogenic material like other chemical fertilizers and are found to improve the soil structure, water holding capacity, microbial biomass, and nutrient availability. Hence, the alternative way for sustainable solid waste management and agriculture.

Fishery is one of the major sectors of agriculture which could solve this problem but damaged a great concern for the management and conservation and environment. India is the second largest supplier of fish in the world after China, with a tremendous 11 fold leap from 0.75 million tons in 1950 to 9.6 million tons by 2012-2013. Nearly 75% of the total weight of the fish was generated as solid waste in the form of gut, head, skin, bones, fines and frames after processing. The fish waste rich in nitrogen, potassium, phosphorus and trace minerals can serve as raw material for the production of many nutritive and nonnutritive products.

II. MATERIALS AND METHODS

For the preparation of fish manure, the collection of fish waste is very important. For present study fish waste was collected from Budhwara Weekly fish market. Fish waste in the form of gut, skin, bones, fins, prawn and crab shells were used as a raw material.

For present study a pit was diggedout having size of 3ft× 3 × 3ft ×3ft. in Science College campus.After preparation of pit, the fish waste was added in pit by layering with soil.

Initially NPK of soil was estimated by standard method and it was noted down. After about 3 months the soil samples from pit were analyzed for NPK in laboratory.

The soil samples from pit were also analyzed from Agricultural laboratory for verification of results.

III. RESULTS AND DISCUSSION

Results of present study are depicted in Table 1 and 2.

Table 1 Availability of NPK in Normal Soil.

Sr. No	Contents	Reading	General Limit
1	pH	7.70	Medium Alkaline
2	Nitrogen	0.80%	High
3	Phosphorous	12kg ha^1	Low
4	Potassium	422.10 kg ha^1	High

Table 2 Availability of NPK in Fish Manure added soil.

Sr. No	Contents	Reading	General Limit
1	pH	7.25	Slightly Alkaline
2	Nitrogen	1.10%	Very High
3	Phosphorous	20.50kg ha^1	Medium High
4	Potassium	429.50kg ha^1	Very High

It is seen from results of present study that; pH has decreased from 7.70 to 7.25, which has reduced alkaline nature of soil from medium alkaline to slight alkaline nature of soil.

Nitrogen content of soil increased from 0.80% to 1.10%. Phosphorous content of soil increased from 12 Kg/ ha to 20.50 Kg/ ha and Potassium content was raised from 422.10 Kg/ ha to 429.50 Kg/ ha.

Therefore fish wastage when mixed with soil after decomposition increases nutrient content of soil. Fish manure can be used for manuring of various types of fish ponds as well as growing of plants. Fish manure is highly nutritious for the growth of plankton in water.

IV. CONCLUSION

It can be concluded from above results that Fish wastage increases NPK of the soil, which enriches the nutrient quality of soil.

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ASSESSMENT OF WATER QUALITY INDEX OF TAKALI LAKE IN PANDHARPUR, DIST: SOLAPUR (M.S.)

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ABSTRACT: *Water quality index is a Simple numerical system to evaluate the suitability or unsuitability of water for various purposes. The present study aimed to evaluate the water quality index (WQI) in order to assess the potability of the water. In the present study quality of water is assessed by using different physico-chemical parameters- pH, TDS, Alkalinity, DO, free Co_2, BOD, phosphate, nitrate chloride, hardness. The physico-chemical parameters were analyzed by using standard method. The water quality index was calculated by using Weighted arithmetic water quality index method. In the present investigation water quality index of Takali Lake was found within the range51.17 to 74.19. It reveals that the water quality of lake is poor. The water quality is deteriorated due to the anthropogenic activities, agricultural runoff.*

Keywords: *Orthoptera, Tettigoniidae, Fauna, Arid region.*

I. INTRODUCTION

Water is the basic need of mankind. There is no substitute for water. Safe drinking water is the necessity of human life (Rakesh*et al*., 2005). Fresh waters of the world are collectively experiencing markedly accelerating rate of qualitative as well as quantitativedegradation(Wetzel, 1983).A body of fresh water which provides a variety of human needs is full of value, only when it is not abused and polluted(Tonapi, 1980). Geometric increase in population coupled with rapid urbanization, industrialization and agricultural development has resulted in high impact on quality and quantity of water (Trivedi *et al.*, 2008).

The term water quality is used to indicate the suitability or unsuitability of water for various uses. Many methods have been developed to interpret the data of water quality assessment. Among all the available methods, water quality indices are the simplest and extremely useful method (Gholamreza, 2015). Water quality indices express the water quality of source in a single value. Water Quality Indices (WQI) is a suitable tool to analyze trends as well as to highlight specific environmental conditions (Abbasi and Abbasi, 2012). The interpretations derived from computing the water quality index are easy to understand for water quality managers as well as general public (Gholamreza, 2015).

The concept of water quality index was first introduced more than 150 years ago in 1848 in Germany. The fitness of the water source was checked on the basis of the presence or absence of the certain organisms in water. Since then different countries have formulated various systems to classify water quality. The first ever modern WQI was developed by Horton in 1965. It heralded a new era because it designed a simple mathematical procedure to integrate physical, chemical, and some biological parameters into a single score (Abbasi and Abbasi, 2012). According to Dunnette (1979) for analysis of water quality index parameters are selected with reference to the oxygen level, eutrophication, health aspects, physical characteristics and dissolved substances. Accordingly the parameters are selected for the present investigation. These parameters are pH, TDS, total alkalinity, total hardness, DO, CO_2, BOD, phosphates, nitrates and chlorides.

II. Materials and Methods

The present investigation is carried out on Takali Lake of Pandharpur city, Dist. Solapur (M.S.). Geographically the lake is located between 17^0 40’ North latitude and 75^0 23’ East longitude. The lake is situated on the South-West of the Pandharpur city and about 465.12 m above mean sea level.

For the present investigation the water samples were collected from three sampling sites of Takali Lake.

Site I – This site is present at West end of the lake. It is situated near Takali village. It lies at $17^0$66’ 69.31” N latitude and 75^o 32’ 63.88” E longitudes.

Site II- This site is the middle area of the lake. It lies at $17^0$66’ 36.48” N latitude and 75^o 32’ 84.55” E longitudes.

Site III- This site is present at East side of the lake. It lies at 17⁰66' 39.15" N latitude and 75⁰ 32' 50.10" E longitudes.

Present investigation is carried out from January 2013 to December 2013. The physico-chemical parameters pH, TDS, Alkalinity, DO, free CO_2, BOD, phosphate, nitrate chloride, hardness were analyzed by following standard methods of APHA (2012) and Ragothaman and Trivedy (2002). The water quality index was calculated by using Weighted arithmetic water quality index method (Brown *et al.,* 1972).

WQI is calculated from the following equation,

$$WQI = \sum qn\, Wn / \sum Wn$$

qn- Quality rating for the n^{th} water quality parameter.

Wn- unit weight for the nthparameters.

According to Weighted arithmetic water quality index method (Brown *et al.,* 1972) water quality index is categorized into 5 categories of water quality.

Table No. 1- Water Quality Index (WQI) Range and Water Quality Status (Brown *et al.,* 1972, Chatterji and Raziuddin, 2002)

Water Quality Index Level	Water Quality Status
0-25	Excellent Water Quality
26-50	Good Water Quality
51-75	Poor Water Quality
76-100	Very Poor Water Quality
>100	Unsuitable For Drinking

III. RESULT AND DISCUSSION

Results obtained in the present investigation are presented in tabulation. Table No.2, 3, 4 represents the calculation of water quality index of Takali Lake at site: I, site: II and site: III respectively. In the present investigation 10 physico-chemical parameters were analyzed to determine the water quality index of Takali Lake.

During the present investigation total dissolved solids were observed in the range 215 mg/L to 355 mg/L. Maximum total dissolved solids, 355 mg/L were observed in summer. In summer concentration of solids in water increases due to decrease in water volume. Alkalinity was observed in the range 105 mg/L to 182.5 mg/L. Maximum total alkalinity, 182.5 mg/L was observed in summer. BOD was observed in the range 3.47 mg/L to 6.2 mg/L. Maximum BOD, 6.2 mg/L was observed in summer. Chlorides were observed in the range 86.52 mg/L to 115.380 mg/L. Maximum chlorides, 115.380 mg/L were observed in summer. The total hardness was observed in the range 116.2 mg/L to 162.5 mg/L. Maximum hardness, 162.5 mg/L was observed in summer. Maximum hardness in summer is attributed to decrease in water volume due to increased temperature.

Water quality index of Takali Lake is calculated for monsoon, winter and summer season. Highest water quality index values were recorded during summer. The water quality index values of summer season ranges from 71.45 to 74.19. Minimum WQI values were recorded in winter. WQI values of winter season ranges from 51.4 to 62.42. The calculated value of water quality index ranges from 51.4 to 74.19. The calculated value of water quality index falls within the range 51-75. This range is classified as poor in terms of water quality categorization (Table No. 1). It clearly indicates that the water quality of Takali Lake is poor. It is unsuitable for human consumption. Poor quality of Takali Lake water is mainly due to agricultural runoff and common human practices.

Maximum values of WQI in summer are attributed to increase in TDS, alkalinity, chlorides and total hardness which is the result of increased pollutant concentration due to the increased rate of evaporation. Similar results were obtained by Yogendra and Puttaiah (2008) Bhadja and Vaghela (2013).

Table No.: 2 Water quality index calculation of Takali Lake at site I

Monsoon				Winter			Summer		
Parameters	V_n	Q_n	W_nQ_n	V_n	Q_n	W_nQ_n	V_n	Q_n	W_nQ_n
pH	7.17	11.666	2.195	7.100	6.667	1.254	7.27	18.330	3.449
TDS	330.00	66.000	0.211	215.000	43.000	0.138	351.250	70.250	0.225
Alkalinity	136.25	113.540	1.512	106.25	135.208	1.801	157.500	131.250	1.748
DO	7.000	79.167	25.325	7.750	71.354	22.825	6.750	81.770	26.157
Free Co2	4.57	20.795	1.512	5.450	24.772	1.801	1.000	4.545	0.330
BOD	5.175	103.500	33.109	3.575	71.500	22.872	6.175	123.500	39.506
Phosphate	2.375	5.278	0.188	1.405	3.122	0.111	1.625	3.611	0.128
Nitrate	2.7	6.000	0.213	1.825	4.056	0.144	2.350	5.222	0.186
Chloride	111.57	44.628	0.285	87.825	35.130	0.225	115.280	46.112	0.295
Hardness	116.2	38.750	0.207	130.000	43.333	0.231	160.000	53.333	0.284
	$\sum W_nQ_n$=64.75			$\sum W_nQ_n$=51.4			$\sum W_nQ_n$=72.31		

Table No.: 3 Water quality index calculation of Takali Lake at site II

Monsoon				Winter			Summer		
Parameters	V_n	Q_n	W_nQ_n	V_n	Q_n	W_nQ_n	V_n	Q_n	W_nQ_n
pH	7.17	11.666	2.195	7.1	10.000	1.882	7.27	18.333	3.450
TDS	332.5	66.500	0.213	216.2	43.250	0.138	350.000	70.000	0.224
Alkalinity	137.5	114.583	1.526	108.750	90.625	1.207	165.000	137.500	1.832
DO	7.02	78.906	25.241	7.72	71.614	22.909	6.750	81.771	26.158
Free Co2	4.35	19.773	1.438	5.67	25.795	1.875	1.02	4.659	0.339
BOD	4.975	99.500	31.829	3.525	70.500	22.552	6.025	120.500	38.547
Phosphate	2.425	5.389	0.192	1.33	2.956	0.105	1.6	3.667	0.130
Nitrate	2.775	6.167	0.219	1.775	3.944	0.140	2.425	5.389	0.192
Chloride	111.680	44.672	2.286	86.52	34.610	0.221	115.380	46.152	0.295
Hardness	117.5	39.167	0.209	131.25	43.750	0.233	161.25	53.750	0.287
	$\sum W_nQ_n$=63.34			$\sum W_nQ_n$=51.26			$\sum W_nQ_n$=71.45		

Table No.: 4 Water quality index calculation of Takali Lake at site III

Monsoon				Winter			Summer		
Parameters	V_n	Q_n	W_nQ_n	V_n	Q_n	W_nQ_n	V_n	Q_n	W_nQ_n
pH	7.25	16.660	3.316	7.17	11.667	2.195	7.3	20.000	3.763
TDS	317.5	63.500	0.203	216.2	43.250	0.138	355.000	71.000	0.227
Alkalinity	132.5	110.416	1.470	105.000	87.500	1.166	182.5	152.830	2.026
DO	7.15	77.604	24.824	7.7	71.615	22.909	7.12	77.864	24.908
Free Co2	4.52	20.560	1.490	5.500	25.000	1.818	1.050	4.773	0.347
BOD	4.925	98.500	31.500	3.475	69.500	22.232	6.2	124.000	39.666
Phosphate	2.45	5.440	0.193	1.425	3.167	0.113	1.7	3.778	0.134
Nitrate	2.725	6.055	0.215	1.850	4.111	0.146	2.45	5.444	0.193
Chloride	111.6	44.660	0.286	86.77	34.710	0.222	115.380	46.152	0.295
Hardness	117.5	39.160	0.209	130.000	43.333	0.231	162.5	54.167	0.289
	$\sum W_nQ_n$=63.7			$\sum W_nQ_n$=51.17			$\sum W_nQ_n$=71.85		

IV. CONCLUSION

The present investigation revealed that WQI of Takali Lake is maximum in summer and minimum in winter. It is found in summer season that WQI values of water were higher. This imparts that water quality of Takali Lake is poor. Agricultural runoff, common human practices such as bathing, washing clothes, cattle washing and grazing are the main reasons for deterioration of lake water quality. To stop further deterioration of lake water there is urgent need of some effective treatment measures.

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ECOLOGICAL STUDIES OF INGLEPAZAR LAKE OF TAHSILPALUS, DISTRICT SANGLI, (M.S.) INDIA

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ABSTRACT: *The present investigation deals with the study of zooplankton biodiversity with physicochemical parameters from Ingle Pazarlake, of TahsilPalus, District Sangli, Maharashtra. The study was carried out for a period of one year i.e. February 2014 to January 2014. During study period, zooplankton and physicochemical parameter were also calculated. Physicochemical parameters such as temperature22.5 to 30.7°C, pH 8.4 to 9.1, electric conductivity 144.2 to 218mg/l, dissolved oxygen 7.7 to 13.3 mg/l, Hardness 75.1 to 177.5 mg/l, Calcium 32.2 to 60.3 mg/l., Biological Oxygen demand 0.6 to 3.6 mg/l , Chloride 62.7 to 109.5 mg/l, Nitrate 5.3 to 26.35mg/l Total solid 64.9 to 167 mg/l. were analysed of the lake water. During the study period total forty three species of zooplankton were identified in four main groups such as nineteen taxa of Rotifera, fourteen taxa of Cladocera,five taxa of Copepoda., five taxa of Protozoa were encountered respectively.Rotifera was the dominant group followed by Cladocera, and Copepod, Protozoa. The high intensity of zooplankton was recorded in summer season and least in winter. Our result indicates that as the productivity of zooplankton was good, it might be continuously utilized for aquaculture, if proper water quality management were mantained.*

Keywords: *Zooplankton, Physicochemical,Rotefera, Cladocera, Copepoda, Protozoa.*

I. INTRODUCTION

The aquatic ecosystems provide habitat to economically and ecologically valuable biological organisms (Ramchandranet., al.2000). The physic-chemical properties of aquatic ecosystems determine the occurrence, abundance and density of fauna.The connection amongst Physico-chemical parameter and zooplanktons inconjunction with the abundance have been calculated, their potential value as indicators of alterations in water quality of lake in this region needs to be assessed. The study of zooplanktons was done in lake to find out the presence growth of zooplanktons. They are vital parts of food chain in the aquatic ecosystems. The freshwater zooplankton comprises Rotifers, Cladocerans, Copepods,Protozoans. The distribution and diversity of zooplankton in aquatic ecosystem depend mainly on the physic-chemical properties of water HarikrishnanK.and Abdul (1989). The physic-chemical parameters and nutrient status of ecosystem play an important role in production of zooplanktons which is the natural food source of many species of carnivorous and omnivorous fishes.(Rahman and Hussain 2008). Thus, the Ingle Pazar lake is now under study of this issue, some studies have started to assess it itchyological practices (Montchowui et al., 2008). It assessed the zooplankton diversity and provided information on the ecological state.

II. MATERIALS AND METHODS:

Study area with geographical location:

The present study is based on the studies carried out for a period of four months from January 2014 to December, 2014. Study area includes, Sangli district. IngalePazar lake is a perennial lake, and important source of water for this lake is rainfall. Geographically it is situated 1705'31.0 N and 74027'29.3''E longitude

Physico-Chemical Parameters of Water

Physico-chemical parameters such as water temperature and pH of water sample were analysed by using a mercury bulb thermometer, pH meter (Model: Digital pH meter) respectively. Dissolved oxygen (DO), Free carbon di-oxide(CO2), Total alkalinity (TA), Calcium, Total hardness, Chloride ,Biological Oxygen demand (BOD) of water sample were analysed by standard titrimetric methods (APHA, 2005).

Collection and preservation of samples:

Zooplankton samples were collected from Three different sites of the IngalePazar lake from January 2014 to December 2014. Samplings were made early in the morning hours 6.00 AM to 8.00 PM. For quantitative analysis, 100 liters of water was filtered through plankton net made up of bolting silk (150µm) to collect zooplankton. These samples were stored in plastic bottles and 4% formalin was added for preservation. These samples were then brought to laboratory for further qualitative and quantitative studies.Quantitative study were made with the help of Sedgwick Rafter cell, identification and enumeration of zooplanktone were done by a light microscope. The systematic identification of zooplankton ws made by

using standared keys od Adoni A.D.(1985), Edmondson W.T.(1963), Pennak R.W.(1968), Dhanapathi M.V. (2000), Altaff K.(2004).

III. RESULT AND DISCUSSION

Table 1 : Monthly variation in physic-chemical parameters in IngalePazar Lake , Palus Jan2014 to Dec. 2014

Parameters	Units	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN
Temperature	C	24.1	26.1	29.0	30.8	24.1	22.5	24.5	23.1	24.1	23.1	24.0	25.1
pH	-	8.48	8.80	9.05	8.73	8.93	8.88	8.93	8.68	8.73	8.95	9.13	8.53
Conductivity		173.75	167.00	195.50	218.00	178.00	166.25	156.75	144.25	146.50	117.25	179.50	180.75
Hardness	Mg/l	89.03	157.25	172.75	177.50	111.50	98.00	75.13	98.65	97.40	80.83	80.18	78.00
Calcium	Ppm	38.53	45.23	52.48	60.33	52.08	45.38	41.05	52.53	48.98	38.58	40.05	35.27
Chloride	Mg/l	98.03	99.78	107.00	109.50	98.55	93.75	87.68	81.03	62.70	78.78	69.65	70.28
BOD	Mg/l	0.65	1.85	2.40	3.63	3.13	2.45	1.83	1.70	2.40	2.05	1.45	0.83
Alkalinity	Mg/l	66.50	78.10	79.35	79.85	79.05	71.53	78.18	78.78	68.85	66.43	55.53	65.25
DO	Ppm	9.93	11..8	12.80	13.30	13.13	12.48	9.95	9.53	8.60	7.70	8.13	9.18
Free CO2	Mg/l	0.28	3.80	4.20	4.82	3.75	4.93	5.20	10.28	11.65	12.00	15.10	3.90
Nitrates	Mg/l	11.50	12.13	20.03	26.35	13.25	27.18	11.60	8.00	6.78	5.30	5.78	9.10
Total Solid		105.50	121.25	147.50	167.03	150.18	128.00	99.75	89.85	75.95	64.98	70.50	89.03

105.50 **121.25** **147.50** **167.03** **150.18** **128.00**

Physicochemical properties of water :

Physicochemical factors such as water temperature, pH, Carbon dioxide, total alkalinity, calcium, are might be responsible for the prevailing population level of aquatic insect in the ecosystem under study are summarized in table 1. In the present investigation the minimum water temperature recorded during month July (22.5^{0}C) and maximum water temperature was recorded in month of May (30.8^{0}C)in water bodies temperature is important parameter of living organisms because of different respiratory rate and metabolism (Devi 2013). The pH concentration has been recorded from February, and it is observed that the specific monthDecember(9.13) .In ecosystem water tempareture and carbon dioxide rate increased rate that caused low pH value (Dubey et.al (2006). DO is essential to all form of aquatic life, the concentration of DO will be resulted as higher in month of May and June (13.3) And the lowest values recorded in month of November (7.7). DO is essential trends in biological activity (Pankow, 1991).

The present study Free CO2 values were considerably maximum in month of December (15.10mg/L) and minimum value was observed in month of January (0.28 mg/L).The total alkalinity was ranges between 55.53- 79.05 mg/L. found in ecosystem. The total hardness found much lower in month of November recorded and the higher range was May. During present investigation the Biological Oxygen demand (BOD) recorded in minimum in month of February (0.65mg/L) and maximum (2.45mg/L), was recorded in July month. The chloride amounts were more observed in 69.65-109.50 mg/L .was recorded. Calcium recorded in higher range was month of May (60.33 mg/L) and lower in month of January.

During summer Temperature, pH, Conductivity, Dissolved Oxygen, Total Hardness, Chloride, Biological Oxygen Demand and Alkalinity, Total Solid was found to be increased and decreased in rainy season. Therefore this lake has rich number of species and biodiversity of aquatic animals.

Zooplanktone in the present study was Rotifera (19 genera), Cladocera(14 genera), Copepoda(5 genera),and protozoa (5 genera).Qualitative and quantitative analysis of different zooplankton are shows in graphical representation.in the present work.Rotiferaconstituent the most dominating group.contributin 50 % to the total zooplankton population followed by Cladocera (39 %), Copepoda (7 %) and Protozoa (4%).

Rotiferans was reported by *Brachionus angularis, B. Calyciflorus, B. caudatus, B.calafertus, B. diversicornis, B.faleatus, B.forficula, B.quadridentata, B.Hexathra sp., Cephalodella sp., Trichocerca Sp.,Filinia terminals, F.longistea, Lacaneluna, L. closterocerca, Keratella testuda, K.quadrata, K .cochleraris, K.tropica*. Rotifera population showed its peak during summer season and after following a decline during monsoon. Maximum population was recorded in winter season due to the less nutrients and dilution of water population of rotifer high during summer season may be availability and abundance of food. Similar

observations were noticed by Sharma (2001), Sampaio et al.,(2002), Hujare (2005)

Cladocera population was second most abundant group of zooplankton and was represented by Alonasp.,Alonapulchella, Bosminia Sp., B.deiteri, B.longirostri, Daphnia vosea, D.longirimis, D.excisum, Monina sp., Monia brachiate, Cypris sp., Cyclocyprisglobosa, Indialonaganapti. Cladocera were high population in summer season, and followed by winter season might be attributed to favourable temperature and availability of food. Copepoda was reported by *Microcyclos sp.,Mesocyclops sp.,* Mesocyclopsleukartiiphyllodiaptomus, Nauplius larva. During summer population of copepod low and high during winter. Similar result have been reported (Yosuf and Qadri (85 and Nyberg 1998), (Rao et al., 2001) The population of Copepoda group were the food source for larval and juvenile fish that link pelagic food web.Copepoda were found to be the consumers of bacteria (Wroblewski,1980) also support the necessary amount of protein source for the aquatic animals. They are mostly carnivorous and feed on the smaller organisms (Gunwati and Mokashe 2014).

Protozoa was represented by Vorticella sps., Paramecium, Aurelia sp., Amoeba radiosa, Chillodenella, metapus. Protozoans play a key role in zooplanktons community and regeneration nutrient role because of their high rates of phosphorous exceretion. Protozoa are high abundance in all aquatic and greatly involved in food web.(Finlay 1997). (Gerald Pfister, 1999).Some species of protozoa are used as valuable bio indicators in quality of water resources, due to their environmental sensitivity. (Ranju R. and JayaprakasV. 2015).

The results clearly indicate that the Rotifera and Cladoceragroups showed high diversity and copepod and protozoa group was showed least diversity values during study period. All the physic-chemical and biological parameters indicate the lake is good in productivity.

IV. CONCLUSION

The study revealed the values of different physic-chemical conditions from the lake. The values did not exceed the desirable limits. A higher value of species diversity and species richness at lake relate the favourable condition in terms of physicochemical conditions and food in the lake. Therefore, conducting further studies in this lake is essential to measuring the diversity of zooplankton and it might be continuously utilized for aquaculture practices.

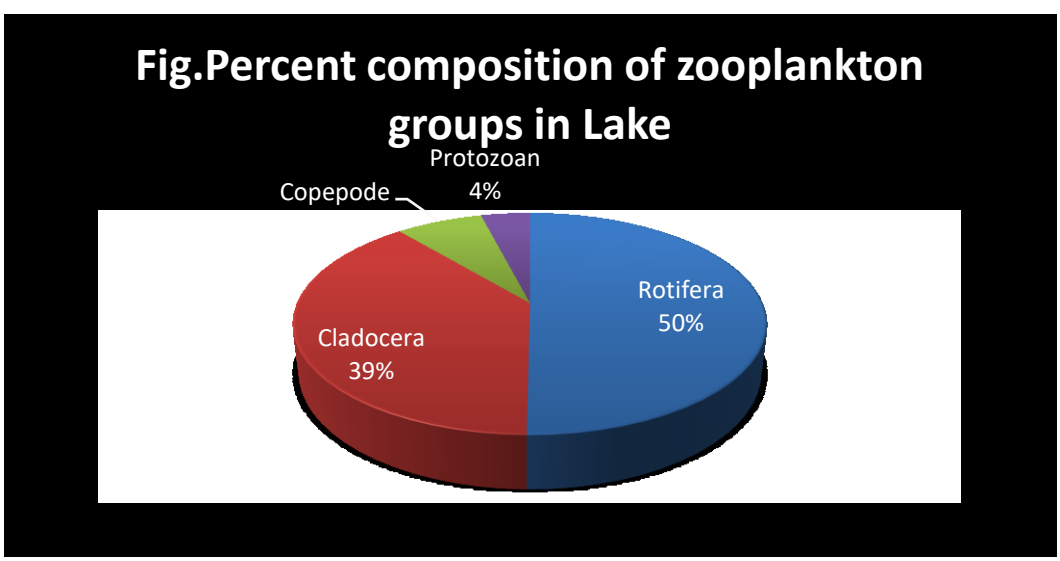

Fig- Distribution of Zooplankton groups in IngalePazarLake.

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INSIGHT TO HERPATOFAUNALDIVERSITY IN AND AROUND MEDHEWADI PERENNIAL WATER BODY OF KOLHAPUR DISTRICT FROM MAHARASHTRA, INDIA

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ABSTRACT: *Present study was carried out so as to reveal the diversity status of herpato-fauna at Medhewadi freshwater body from Maharashtra. The study was carried out by using visual survey technique. The investigation revealed 10 species of amphibians, among which 9 were anurans and 1 was caecilian while 12 species of reptiles, among which 3 species of lizards and 9 species of snakes were recorded. Amphibians demonstrated Dicroglossidae as dominant family; on the other hand reptiles exhibited Colubridae as dominant family. The investigation also revealed that there was considerable variation in utilization of micro-habitats by the both amphibians and reptiles. The study can be concluded that area of this freshwater body is medium rich in herpatofauna.*

Keywords: *Herpatofauna, Medhewadi, perennial, water body, Kolhapur District.*

I. INTRODUCTION

Freshwater reservoirs are important aquatic habitats as they provide required ecological conditions to many plants and animals. These sites accommodate many invertebrates, fishes, amphibians, reptiles, aves and mammals. Hence, complex ecosystem is formed at aquatic habitats. Though, amphibians and reptiles are not totally dependent on aquatic habitats, they spend their life stage in the aquatic habitat, especially, larval stages of frogs. However, reptiles also have a better attachment with wetlands in search of food. Recently and in past many researchers were concentrated on the study of herpatofauna at various parts of the world but study on wetlands is very much less. Hence, present study was focused on the herpato-faunal diversity at a small wetland of Kolhapur district.

II. MATERIALS AND METHODS:

STUDY AREA:

Medhewadi is a small village in the Ajara tehsil of Kolhapur district where percolation tank was constructed by Irrigation Department. The GPS location of this reservoir is 16^0 07' 368" N and 74^0 16' 952" E with the total submergence are of 2.93 ha. (Patil, 2015)

DIVERSITY ANALYSIS:

Amphibians are sensitive creatures of the universe and need more attention. The present study was carried out in the monsoons of the years 2011-12 to 2014-15. During the study period, frequent visits were made to reservoirs and their adjoining areas to collect amphibians through visual survey technique (Fellers and Freel, 1995). Collected specimens of amphibians were identified on the field by using standard references, viz. The book of Indian reptiles and amphibians (Daniel, 2002) and Pictorial guide to frogs and toads of Western Ghats (Gururaja, 2012), photographed (using Canon 600 D Camera with 18-55 mm lens) whenever possible and collected specimens of amphibian were released back to collection point.

III. RESULT AND DISCUSSION:

Medhewadi freshwater reservoir exhibited 10 species, since 9 were anurans and 1 was caecilian (Table 1). Family wise composition is as shown in Figure 1. The total species of amphibians are mainly belonging to 11 genera and 6 families viz. Bufonidae, Dicroglossidae, Microhylidae, Ranidae, Rhacophoridae and Ichythyopidae. Between these, Dicroglossidae and was dominant with 36.36 %. Thesetaxons were noticed from diversified microhabitats and categorized as terrestrial and burrowing, arboreal, semiaquatic and aquatic on the basis of their habitats. Mostlythese amphibians were cited in the months of rainy season while some of them were cited immediately after monsoon and in the post monsoon season. The genera like *Duttaphrynus*and *Sphaerotheca*were terrestrial and burrowing and observed at the adjoining region. One of the species belonging to *Euphlyctis* was aquatic,threespecies (*Polypedatesmaculatus, Roarchestesbombayensis and Limnonectuslimnocharis*)were arboreal while remaining were semi-aquatic.The arboreal species were observed from the shrubby aquatic vegetation at the adjacent places of this reservoir

while semi-aquatic species were recorded from the water as well as the adjoining region. Among all, the species, *Hoplobatrachus was* commonly observed. One species of *Ichythyophis*was also noted from this site.

According toVencesand Kohler (2008), of the total of 5,828 amphibian species considered here, 4,117 are aquatic in that they live in the water during at least one life-history stage, and a further 177 species are water dependent. Hence, study was carried out to and it revealed that the amphibian diversity was relatively rich Medhewadi freshwater reservoir. The upstream of this reservoir is covered with canopy which ultimately reduces the light penetration. This might help amphibians to remain moist and active at daytime also and increased the rate of sightings. Ganesh *et al.* (2007) have also noted similar observations in the rain forests of Karnataka. However, physicochemical characteristics and anthropogenic activity might influence the diversity of amphibians. Medhewadi water body faces medium level of human activities. All over the world, no exclusive work is noted with reference to diversity status of amphibians in particularly wetlands or water bodies. The study also reveals that, Dicroglossidae was dominant over all other families; this might be due to the habitat selection. Most of the species belonging to this family prefer leaf litter, streamside, dam backwater, near water bodies, lakes pools etc. These results coincide with Gururaja (2010) in pictorial guide to frogs and toads of the Western Ghats.Moreover, species belonging to this family is either semi-aquatic or aquatic in habitat.

The investigation also emphasized that Medhewadi freshwater reservoir exhibited 13 species of reptiles (Table 2) belonging to 6 families among which coloubridae was dominant family, representing 6 taxa, with 46.15% of the total diversity (Figure 2).

There were 3 species of lizards recorded, belonging to three genera and two families, viz. Agamidae and Scincidae. Agamidae was represented by two species, Roux's forest lizard and Indian garden lizard while Scincidae exhibited only one species as bronze grass skink.

The study site demonstrated a quite rich diversity of snakes with a total number of 10 species of snakes belonging to 12 genera and 4 families. Among all families Coloubridae was dominant. The five families recorded during the investigation period were as Uropeltidae, Coloubridae, Elapidae and Viperidae. However, study also revealed that 3 species were venomous, 1 was semi-venomous and 6 were non-venomous.

Indian rat snake was encountered commonly during study period. Common Kukri snake was also encountered at this site. Three species of keelback, viz. checkered keelback, stripped keelback and green keelback were recorded at this site; among which checkered keelback was the most common while later two were commonly encountered. One species of vine snake was observed, viz. Green vine snake that was commonly found during the period of study. One species of Trinket snakes (Common trinket) was recorded. During the study period, three species of poisonous snakes were encountered representing two families, viz. Elaphidae (common krait and spectacled cobra) and Viperidae (Russell's viper).

IV. CONCLUSION

On the basis of present investigation, amphibian and reptilian diversity is quite rich and further conservation measures must be employed in future so as to increase the richness of diversity profiles.

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ZOOPLANKTON ABUNDANCE AND COMPOSITION IS NOT SAME IN ALL SEASONS OF AYEAR IN SAME LAKE

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ABSTRACT: *The quantity and quality of lake water is not same throughout the year. In summer season Kurnur reservoir in Solapur district of Maharashtra is almost dry with patches of water having algal boom. Variations in zooplanktoncomposition and abundance were studied with respect to seasonal changes in physical and chemical properties ofdam water from January to December 2014.Zooplankton composed of Rotifera,Copepoda, Cladocera, Ostracoda and Protozoa. In summer season 24 species of zooplankton were identified, in rainy season 16 and in winter season 15 species were identified.Rotifera dominated in all seasons of the year followed by Copepoda, Cladocera, Ostracoda and Protozoa. In summer season abundance of Rotifera is 62 %Copepoda (15%), Cladocera (14%),Ostracoda (6%) and Protozoa (3 %) of the total population. Abundance of zooplankton changed in winter and rainy season. In rainy season abundance of Rotifera is(47%), Copepoda (20%), Cladocera (17%), Ostracoda(11%) and Protozoa (5%). In winter season zooplankton composition and abundance is almost same as that of rainy season with slight variations. In winter season abundance of Rotifera is(49%), Copepoda (22%), Cladocera (15%), Ostracoda (9%) and Protozoa (5%). AmongstRotifera species Brachionusforficula, Brachionusquadridentatus,Brachionusangularis and Trichocerca species dominated insummer season which are indicators of pollution.*

Keywords: *Zooplankton, freshwater reservoirs*

I. Introduction

Lakes, reservoirs and rivers are the important sources of freshwater. In world, most of the human settlements are associated with water reservoirs. The quality of water influenceover organisms found within the reservoir as well as surrounding to it including human beings.Healthy composition of zooplankton in the reservoir is indicator quality and healthof aquatic ecosystem. Zooplankton are heterotrophic organisms, consume phytoplankton and grow in number as well as size. They are consumed by small fishes, larva, tadpoles etc. The large fishes feed on small fishes or larva and complete the food chain. As small fishes, larva and tadpoles are the food of large fishes, therefore the fish production is associated with plankton production(Ryder et al., 1974). The quality parameters of water are very much influential over diversity and abundance of zooplankton. Physical and chemical properties of water are responsible for quality and abundance of phytoplankton and zooplankton(Odum et al., 1971). Seasonalvariation in amount and duration of rainfall, agricultural runoff, kind of agricultural practices in catchment area, domestic sewage discharge and industrial effluents are responsible for variation in physical and chemical properties of water.

Zooplankton community include Rotifera, Copepoda, Cladocera and Ostracoda and Protozoans. The community structure means, arelative composition and abundance of zooplankton at a specific period or time. The composition and abundance of plankton species depends onrelative range of tolerance towards changing seasonal physical and chemical properties of water. The zooplankton abundance is not same throughout the year. The question arises which of physical and chemical factors influence the fluctuation in plankton population, how the zooplankton sustain their life in limited amount of water during summer drought, how they co exists. According to Huisman abundances of planktons allow the coexistence of many species on a handful of resources (Huisman and Weissing 1999).

During summer season due to lack of water pollutants from drainage from nearby villages accumulate in patches of water which lead to pollution of water. The polluted water has different composition of zooplankton in comparison to non polluted water. Appearance of few Rotifera is indicative of pollution. The relative composition and abundance of zooplankton isconsidered as indicators of pollution. In a study at Sadatpur reservoir *Sinantherina species,Rotaria* and *Asplanchuna* are found relatively abundant which is indicator of water pollution (Avinash B. Gholap, 2014). Few members of rotifer when present in abundant number, are indication of eutrophication of lakes, for example *Brachionusforficula, Brachionusnilsoni*, and *Trichocercas*p(AzmaHanim Ismail et.al, 2016).In contradictory to that in one of a study at lake ParqueAtalaia in America, Rotifera diversity was markedly low during dry season under the

influence of pollution of water by inlet of domestic sewage (Neves. et.al., 2003). From these study it is clear that zooplankton are indicators of pollution and quality of water.

The objective of this research is to find out whether seasons influence the zooplankton community structure, if yes then what are the reasons for that in other words which of the physical and chemical factors are responsible for that.

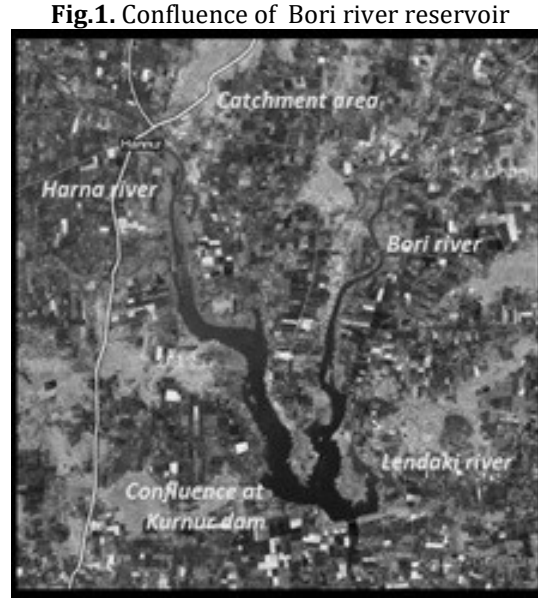
II. MATERIALS AND METHODS

Kurnur Dam isnine gated earthen dam, located at 17°37'0"N latitude and 76°13'2"E longitude.It is constructed at confluence of Harna and Boririver. The dam covers the catchment area of 1,254 km2 from Akkalkot and TuljapurTehasil. Dam is located in drought prone area and rain shadow region of Western Ghats of Maharashtra. The google map of dam is attached in Fig. No.1.The quantity of water in the reservoir depends upon monsoonal rainfall in its catchment area.

The samples were collected according to standards and procedure for examination of water and waste water as published in American Public Health Association (APHA-1989) as well as 10th and 17th edition of Burro of Indian standard methods of sampling and test for water and waste water (BIS-3025) which was used as a manual for water analysis. Water samples were collected at confluence of Harna, Bori and Lendakiriver through suction pump method at different fixed spots on every alternate Sunday from January 2014 to December 2014. The field parameters which are essential to be measured immediately like temperature, pH, conductivity and nitrate wereimmediately collected at site or spot only. Chemical parameters were analysed in laboratory of Department of Zoology, Sangameshwar College, Solapur. These chemical parameters were total hardness, turbidity, TDS(Total Dissolved Solids) BOD, COD etc. Cations and anions include Ca, Mg, Sulphates, Nitrate, Phosphate were analysed as per procedure mentioned in USGS manual and EPA government manuals (USGS Manual and eps.gov) (epa.gov manual).

Zooplankton were collected with help of plankton net of mesh size 325 (44 micron size of eye)by pumping 50litters of water from different strata and immediately preserved with 4% formalin mixed with borax powder. Zooplankton were analysed with the help of Sedgewick Rafter counting cells. Zooplankton were identified with help of taxonomic key (Idris 1983) to the lowest possible extent. Physical and chemical parameters were correlated with abundance of zooplanktonwith help of statistical applications. The data of relative abundance and composition is express in graphical presentation for tangible understanding.

Fig.1. Confluence of Bori river reservoir



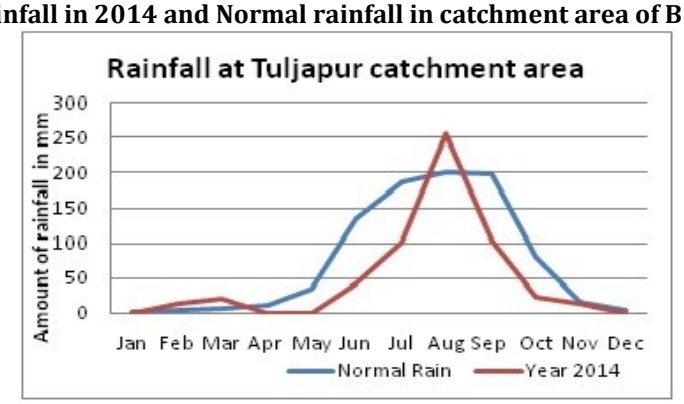
III. RESULT AND DISCUSSION

In 2014, there was better rainfall as compared to normal rainfall in the month of March and August, both of these months are marked by different agricultural activity in catchment area. The good amount of rainfall from western disturbance locally known as '*Awakali* ' in the month of February (13.9 mm) and March (19.9 mm) in catchment area almost made the reservoir full (Fig.No.2). This reduced the drought

effect in the month of April and May as it happened every year earlier. The good amount of rainfall intensified the agriculture activity in the catchment area, frequent tilling, use of fertilizers and pesticides that dissolved in agricultural runoff and flowed in to the reservoir. There is no industrial belt in the catchment area so no question of industry effluent related pollution but some settlements are present on the bank of Harna river which is the main source domestic sewage and pollution of reservoir water.

The samples were collected on every fourth Sunday, thus all the parameters show double reading in the month of September. The data are classified into seasons. Month of November, December and January climate is relatively cold so considered as winter season. February, March, April and May are having high temperature so considered as summer season and June, July, August, September and October are considered as rainy season of monsoonal rainfall.

Fig.2 Rainfall in 2014 and Normal rainfall in catchment area of Bori river.



The physical and chemical parameters of dam water are mentioned in Graph no 1 to 8. These graphs are based on data collected from January 2014 to December 2014. Graph no.1 represent values for Temp, pH and turbidity of water in summer, rainy and winter season. Temperature is recorded highest in the month of June 31^{0}C and lowest in December 19 ^{o}C. The annual range of temperature is high. Summer is warm with average temperature of water 26 ^{o}C, rainy season is warm and humid while winter temperature is average 20 ^{o}C. Turbidity of water is high in July (12.6 NTU) and August (15.6 NTU) rainy season, which associated with agricultural practice and increased run off carrying more particles with high amount of rainfall as compared to other seasons. In winter season water is almost less turbid. Turbidity is almost same throughout the year except in rainy season. The dam water is basic in nature having average pH of 7.6. In rainy season pH is lower 7.1 and summer season pH is higher 7.9. The lowest value pH of water is also basic in nature (7.1). It means the dam water has no acidic pH but have basic pH throughout the year.

In Graph No. 2 electric conductivity is measures in term of mmho/cm. Conductivity is associated with addition of water to the reservoir in rainy season. It shows two peaks one in April (522.15 mmho/cm) and other in August (687.34 mmho/cm). The March peak is associated with *Awakali* rainfall which is smaller in amount as compared to monsoonal rainfall in August. Therefore conductivity is higher in rainy season and lower in winter and summer season.

Graph No. 3 represents all data related oxygen like Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). COD has wide range of fluctuation in comparison to DO and BOD, which means oxidation of organic compound is more summer i.e in the month of March and April, when water is limited to small patches. COD is measure of organic pollution. In summer season addition of domestic sewage from Kurnur village increase COD level of water. Dissolved Oxygen is higher August (7.53 mg/L) and September (7.16 mg/L) which is associated with inlet of rain water through different rapids from watershed area. Dissolved Oxygen is lowest during winter season when there is high BOD and no addition of DO by inlet of water. In winter available oxygen is consumed by organisms for the metabolic activity. Dissolved oxygen is also low in summer because high COD.

Graph No. 4 measures Nitrogen level in water. Nitrogen exist in three forms Nitrate (NO_3), Nitrite (NO_2) and Ammonia (NH_3). In our study Ammonia is not considered for study. Nitrate (NO_3) is volatile and rapidly consumed therefore it should be analysed very quickly with the help of kit. Nitrite (NO_2) level is high in summer and winter and less in rainy season. Nitrite (NO_2) is maximum in May (0.33 mg /L) which is associated with pollution in water patches. The range of Nitrite (NO_2) fluctuation is less as compared to Nitrate (NO_3). Nitrate (NO_3) is more in amount in rainy season in the month of August (1.6 mg/L) and

September (1.44 and 1.22 mg/L) which is associated with inlet of water through agriculture runoff. It means Nitrate is contributed by the agricultural fertilizers that farmer use to enhance their crop. The graph indicated Nitrate (NO_3) has high fluctuation range that is maximum in rainy season and minimum in winter and summer season. Similar results were found in a study of Ujani dam (Kimbhar A C et al., 2006).

Graph No. 5 Measures hardness of water which is summation of calcium and magnesium concentration. Calcium and magnesium concentration increase in rainy season and decrease in winter and summer season. It means the runoff water dissolve surface calcium and magnesium and bring it to dam water. Calcium level is high in August (246.43 mg/L) and September (233.57 and 221.63 mg/L), and the same case with magnesium also August (183.45 mg/L) and September (173.2 and 164.52 mg/L). Therefore water is more hard in rainy season as compared winter and summer season. Total hardness of water in August (429.88 mg/L) and September (406.77 and 387.19 mg/L) are high in comparison to December (279.54 mg/L) winter season. In a study of seven different lakes in similar fluctuation in calcium, Magnesium and total hardness level are observed (Thangamalathi et al., 2018)

Graph No. 6 represents Phosphate, Sulphur and Chlorine level in dam water. The surrounding economic activities are responsible for contribution of these minerals in dam water. Use of sulphur and phosphate in fertilisers dissolve in agricultural runoff and enters the dam water. The chlorine is contributed by tar used in construction of roads in the catchment area, use of bleaching powder that is dissolved in domestic sewage etc. Even though Phosphate and Sulphur is contributed by the agricultural runoff their concentration is more in summer season rather than rainy season. It is because of the rate of utilization of these minerals by organisms do not match with rate of influx. Therefore they accumulate with evaporation of water and diluted with inlet of water. Phosphate content is more in April (2.58 mg/L) and May (2.68 mg/L) summer season while diluted in rainy season in August (0.92 mg/L) and September ((1.11 mg/L) in rainy season. There is little dilution effect found in March (2.3 mg/L) due to *Awakali* rains. The average sulphur concentration is (15.45 mg/L). From the graph it seems that Sulphur concentration gradually increases from January winter to summer and to rainy days of July and August, reaching maximum in August (19.33 mg/L) and start to decline gradually by winter. It means there is limit to its inlet through agricultural runoff after that inlet of rain water dilutes it. The concentration of chlorine shows two peaks one in March (18.58 mg/L) and other in August (38.09 mg/L). It means more the inlet of water more the its concentration.

Graph No. 7 represents suspended and dissolved solids. Total dissolved solids (TDS) and total suspended solids (TSS) are maximum in rainy season which is associated with energy in flowing water. In rainy season the soil particles dissolve in water and some of them are carried in suspended form. In August TDS is 558 mg/L and TSS is 23.49 mg/L and in September TDS is 528 mg/L and TSS 23.18 mg/L which are maximum in as compared to summer and winter season. In December TDS is 364 mg/L and TSS is 13.12 mg/L. Summer season has little higher TDS and TSS as compared to winter season. Total Solids (TS) is summation of TDS and TSS. TS is highest in August 580.93 mg/L and September 551.51 mg/L and lowest in December 377.39 mg/L

Graph No. 8 represents Sechi depth, that is associated with transparency of water. The transparency is more in undisturbed and unpolluted water. In rainy and summer season Sechi disc cant be viewed from far depth due to turbid water. Sechi depth is maximum in January 98.2 cm and February 94.66 cm and minimum in September 54.4 cm because of turbid water in rainy season.

Zooplankton include Rotifera, Copepoda, Cladocera, Ostracoda and Protozoa. Totally 41 planktons were studied including protozoa's. Rotifera 18 species, Copepoda 6, Cladocera 6, Ostracoda 5 and Protozoa's 5 as mentioned in Table No. 1, 2, 3 , 4 and 5.

Table. 1. Rotifera species found in collected water samples in 2014 at Kurnur dam

Brachionus calyciflorus	*Keratella quadrata*	*Euchlanis dilatata*
Brachionus caudatus	*Keratella tropica*	*Filinia longiseta*
Brachionus falcatus	*Lecane bulla*	*Filinia spp*
Brachionus angularis	*Lecane pyriformis*	*Testudinella sp.*
Brachionus forficula	*Monostyella sp.*	*Trichocerca tigris*
Brachionus quadridentatus	*Notholca acuminata*	*Rotaria spp*

Table. 2 Copepoda larvae species found in collected water samples in 2014 at Kurnur dam

Mesocyclop sps	*Diaptamus spp.*	*Cyclops viridis*
Nauplius larvae	*Paracyclops spp.*	*Mesocyclops leuckarti*

Table. 3 Cladocera species found in collected water samples in 2014 at Kurnur dam

Alona spp	*Daphnia sp*	*Bosmina*
Alona rectangula	*Moina mircura spp*	*Ceriodaphnia pulchella Sars*

Table. 4 Ostracoda species found in collected water samples in 2014 at Kurnur dam

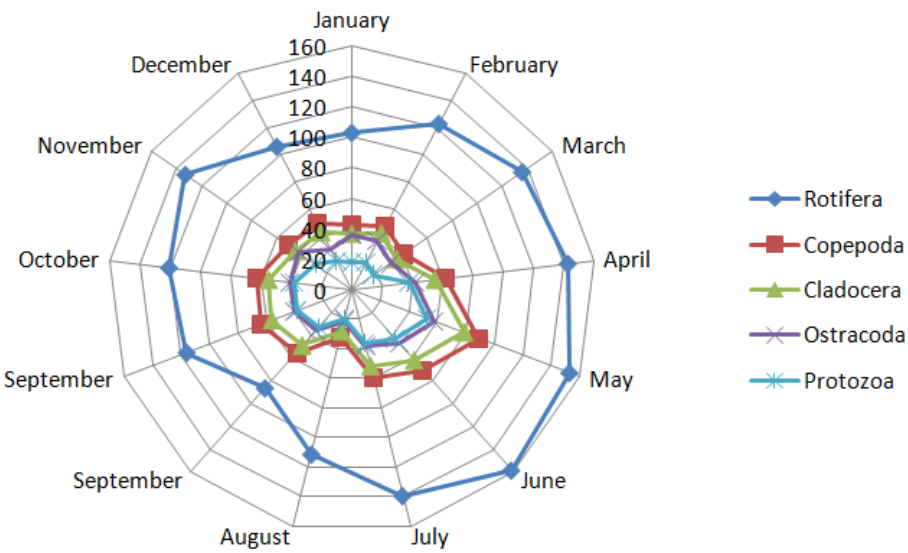
Candocypris spp.	*Cypris spp.*	*Stenocypris spp.*
Centrocypris	*Metacypris*	

Table. 4 Protozoa species found in collected water samples in 2014 at Kurnur dam

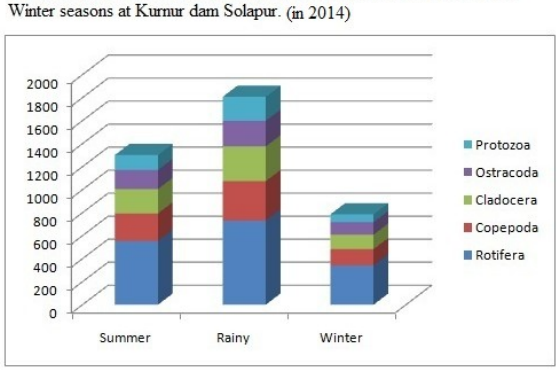
Amoeba	*Arcella*	*Diffluga spp.*
Paramoecium	*Euglena*	

Rotifera dominated the zooplankton community in all seasons followed by Copepoda, Cladocera, Ostracoda and Protozoa's as mentioned in figure no 3. In summer season March, April and May percentage of Rotifera is high in community composition. In all seasons Protozoan count is low which may be because of escape ability.

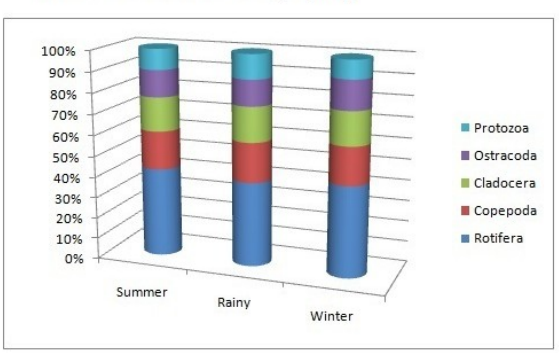
Fig.No.3 Abundance and composition of zooplankton in all seasons of 2014 at Kurnur dam



Dig.4 Composition and abundance of zooplanktons in Summer, Rainy and Winter seasons at Kurnur dam Solapur. (in 2014)



Dig.5 Percentage composition of zooplanktons in Summer, Rainy and Winter seasons at Kurnur dam Solapur. (2014)



In summer season 24 species of zooplankton were identified, in rainy season 16 and in winter season 15 species were identified. Rotifera dominated in all seasons of the year followed by Copepoda, Cladocera, Ostracoda and Protozoa. In summer season abundance of Rotifera is 62 % Copepoda (15%), Cladocera (14%), Ostracoda (6%) and Protozoa (3 %) of the total population. In winter and rainy season abundance of zooplankton changed. In rainy season abundance of Rotifera is(47%), Copepoda (20%), Cladocera (17%), Ostracoda (11%) and Protozoa (5%) as in fig. No.5. In winter season zooplankton composition and abundance is almost same as that of rainy season with slight variations. In winter season abundance of Rotifera is(49%), Copepoda (22%), Cladocera (15%), Ostracoda (9%) and Protozoa (5%) as in figure no. 4 and 5. Amongst Rotifera species *Brachionus forficula, Brachionus quadridentatus,Brachionus angularis and Trichocerca species* dominated insummer season which are indicators of pollution.Highest number of Rotifera are counted in warm months May153 org/L and June158 org/L. It coincides with temperature rather than any other factors. It means temperature is most important factor that govern reproductive ability of Rotifera. In rainy season their count was less i.e September 86 org/L which is because of dilution effect of influx of new water from rainfall in catchment area. The same thing happens with Copepoda and Cladocera. In May Copepod count is 89 org/L and Cladocera count is 79 org/L while in June they are respectively 70 org/L and 62 org/L which is quite high in comparison to December and January count. In December Rotifera 106 org/L, Copepoda 49 org/L, Cladocera 43 org/L and Ostracoda 30 org/L. From the fig.No.3 It is quite clear that Summer season is favourable for growth of all the planktons and winter season is unfavourable. There is wide range of fluctuation in community composition especially for Rotifera. Zooplankton composition indicates species richness that is number of species occurred and abundance of zooplankton means number of individuals population of each species. In one of the study it was found that species richness was inversely related to abundance, as species richness was highest in summer season while abundance of plankton was highest in rainy season. In rainy season Cladocera dominated and in summer season Rotifera dominated. This was the study made in Nigerian floodplain by Okogwu (Okogwu OI1, 2010).

If the planktons community composition is correlated with the a biotic factors, it is found that Rotifera population show strong and positive correlation with temperature, but weakly positively related with pH, BOD, COD, NO_2, Phosphate and Sulphate concentration. Rotifera are negatively correlated with electric conductivity, turbidity, TDS,TSS,TS, DO, NO_3, Ca,Mg,TH and Cl. Copepoda and Cladocera are not correlated with pH. Copepoda and Cladocera is correlated strongly and positively with temperature and weakly positively correlated with phosphate and sulphate concentration. Ostracoda is strongly and positively correlated with temperature and weekly positively correlated with pH, Sechi depth, BOD, COD, NO2 and Phosphate. While strongly negatively correlated with suspended solids. Protozoa's are strongly positively correlated with temperature and weakly positively correlated with electric conductivity, calcium, and magnesium, phosphate and sulphate concentration.

Conclusion.

Temperature is the most important factors that govern the zooplankton community composition. Number of Rotifera, Copepoda, Cladocera and Ostracoda are more in water samples in summer season. Other factors are also influential either negatively or positively. In summer season water is found in patchy places with almost greenish appearance. The accumulation of pollutants in small quantity of water justifies the increased number of *Brachionus forficula, Brachionus quadridentatus,Brachionus angularis and Trichocerca species*Rotifera species.

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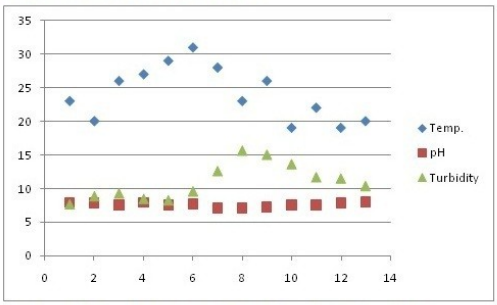
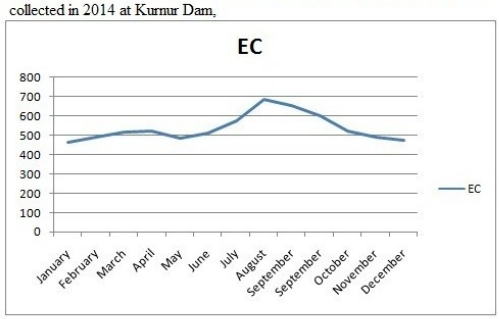
Graph 1 Physico-chemical parameters Temperature. pH and Turbidity of water samples collected in 2014 at Kurnur Dam,

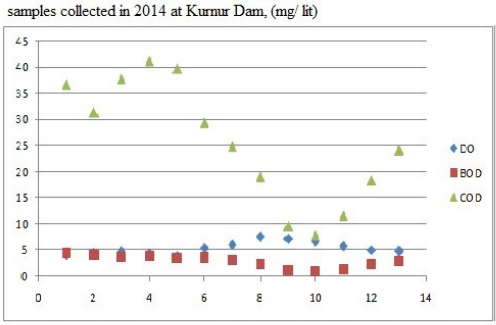


*1-January, 2- February, 3- March, 4- April, 5- May, 6- June, 7-July, 8- August, 9- September, 10- September. 11-October. 12- November. 13-December

Graph 2 Physico-chemical parameters electric conductivity of water samples collected in 2014 at Kurnur Dam,

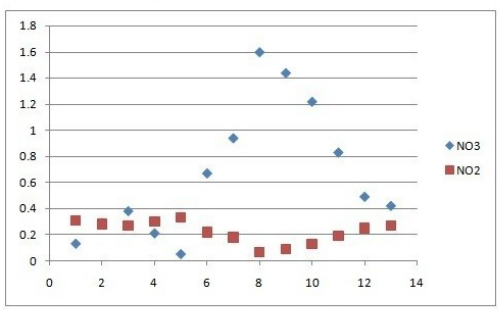


Graph 3 Physico-chemical parameters Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of water samples collected in 2014 at Kurnur Dam, (mg/ lit)



*1-January, 2- February, 3- March, 4- April, 5- May, 6- June, 7-July, 8- August, 9- September, 10- September. 11-October. 12- November. 13-December

Graph 4 Nitrate and Nitrite concentration in mg/ lit of water samples collected in 2014 at Kurnur Dam



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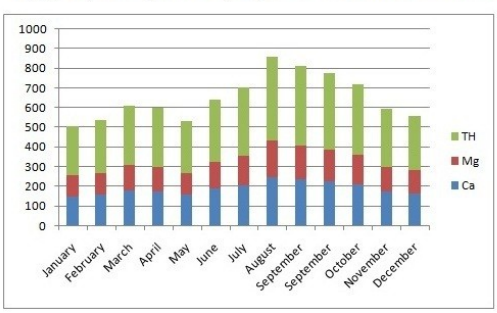
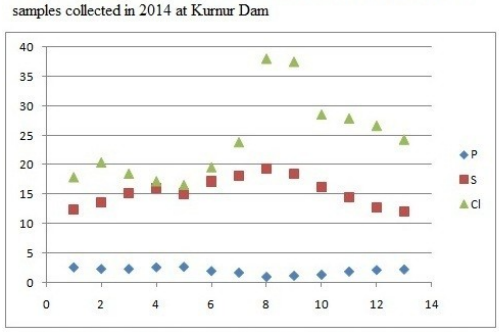
Graph 5 Physico-chemical parameters Total Hardness, Calcium and Magnesium Concentration of water samples collected in 2014 at Kurnur Dam

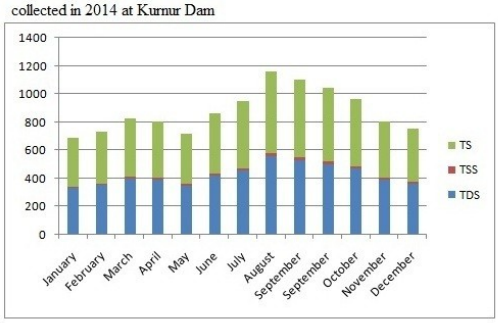


Graph 6 Phosphate, Sulpher and Chlorine concentration in mg/ lit of water samples collected in 2014 at Kurnur Dam

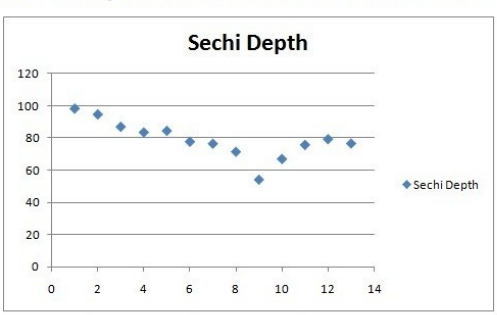


*1-January, 2- February, 3- March, 4- April, 5- May, 6- June, 7-July, 8- August, 9- September, 10- September. 11-October. 12- November. 13-December

Graph 7 Physico-chemical parameters Total Dissolved Solids (TDS), Total Suspended Solids (TSS) and Total Solids (TS) in mg/ lit of water samples collected in 2014 at Kurnur Dam



Graph 8 Sechi depth in centimeters of water collected in 2014 at Kurnur Dam



*1-January, 2- February, 3- March, 4- April, 5- May, 6- June, 7-July, 8- August, 9- September, 10- September. 11-October. 12- November. 13-December

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NEPHROPROTECTIVE EFFECTS OF ETHANOLIC EXTRACT OF *A. PANICULATA* ON THIOACETAMIDE TOXICITY IN RATS

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ABSTRACT: *The present work was conceded out to check nephrtoprotective potency of ethanolic extract of Andrographis paniculata. The whole plant extract of A. paniculata was used for the treatment of toxicity induced in the kidney of albino rat, Rattus norvegicus by using thioacetamide (TAA). Thioacetamide toxicity in the animal was manifested by the significant increase in the level of serum creatinine, blood urea nitrogen (BUN), serum sodium and serum potassium. Administration of ethanolic extract of A. paniculata doses (250 mg/kg b. w). showedthe significant decrease in the level of serum creatinine, BUN, serum sodium and serum potassium when compared with induced rats. These results thus proved the potential nephroprotective effect of ethanolic extract of A. paniculata.*

Keywords: *Thioacetamide, nephrotoxicity, A. paniculata, Protective effect*

I. Introduction

Kidney is vital organs that play an important role in homeostasis by excreting the metabolic waste products depending on the needs of the body (Hall, 2011). It is mainly susceptible organ to the toxic injuries because of high blood provide and presence of cellular transport systems that causes gathering of these compounds within the nephron epithelial cells (Faller and Gstraunthaler, 1998). Acute kidney damage are the most common and serious health difficulty having high morbidity and mortality rate (Begum *et al*., 2006).

Thioacetamide is one of such toxic chemical used as a fungicide, chemical reagent, organic solvent, accelerator in the vulcanization of rubber, paper, dye, textile and as a stabilizer of motor oil (Chen *et al.* 2008; Lee *et al.* 2003). Thioacetamide form toxic effect due to formation of thioacetamide-S-oxide which reacts with proteins resulting into denaturation. The administration of TAAcauses cell death by necrosis in addition to apoptosis in experimental animals (Li-Hsuen et al. 2006).

The herbal medicines have recently attracted great attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is obtainable about their mode of action(Singh and Singh, 2016). The medicinal plants are the source of the excess of bioactive compounds, exploited for natural product based drug development program for the curing of many diseases (Singh *et al.* 2014).

A. paniculata is an herbaceous plant commonly called as 'King of bitter in the family acanthaceae. *A. paniculata* is a medicinal plant conventionally used for the treatment of cold, fever, laryngitis and numerous infectious diseases ranging from malaria to dysentery and diarrhea in china, India and south East Asian countries (Siddhartha and Rajender, 2007). Itcommonly known as 'kalmegh' in India, and is used as a bitter ingradient in 26 ayurvedic formulations as antiangiogenic (Sheeja*et al*. 2007), immunomodulatory (Xu*et al.* 2007), anticancer (Kumar *et al.* 2004) and used for treatment of various liver disorders (Handa*et al.*1986). Therefore, in the present study was designed to investigate the ethanolic extract of effect of whole plant of *A. paniculata* against thioacetamide induced nephrotoxicity in albino rats of either sex.

II. MATERIAL AND METHODS

2.1 Animals

The healthy adult albino Wistar rats (130 – 150gm) were procured from Hindustan Antibiotic Ltd., Pune and they were acclimatized in laboratory conditions at Rajarambapu College of Pharmacy, Kasegaon for about two weeks (Regi. No. 209/CPCSEA).They were fed with Amrut rat feed obtained from Pranav Agro Industries, Pvt. Ltd., Sangli and water ad libitum. All the experimental procedures were carried out by accordance to the guidelines of Institutional Animal Ethics Committee.

2.2 Plant Material

A. paniculata is annual herbaceous plant from family acanthaceae. It grows erect upto a height of 30-110 cm. in moist shady places. The root is tap root and stem is slender, dark green in colour and squared. The leaves are simple, glabrous, lanceolate, hairless blades measuring of about 4 – 12 cm. long and 1 – 3 cm wide. The flowers are small, white with rose purple spot onthe petals. The flowers are scattering axillary

and terminal racemes. The fruits are capsule about 2 cm. long and it contains yellow brown seed. Flowering and fruiting time is between May to October.

3.1 Preparation of Injectable solution of drug

Injectable solution of TAA (Sigma Aldrich, Switzerland) was freshly prepared by dissolving TAA crystals in sterile and distilled water with constant stirring until all crystals were dissolved. The injectable TAA solution (200 mg / kg body weight) was administered intraperitoneally (i.p.) to rats thrice a week for about 8 weeks (Alshawsh et al., 2011).

3.2 Collection of plant material and extraction

3.2.1 Collection of plant material

A. paniculata plant was obtained from Botanical garden of Krishna Mahavidyalaya Rethare BK. It was identify and authenticated by Dr. C. B. Salunkhe, Department of Botany, Krishna Mahavidyalaya Rethare BK. The voucher specimen of *A. paniculata*(Collection No. KMR 1719)has been kept in our laboratory for future reference.

3.2.2. Preparation of extracts

Freshly collected whole plant material of *A. paniculata* was collected, washed and shed dried for about 45 days and which was subjected to maceration to form crude powder was used for extraction. The powdered plant material of *A. paniculata*was extracted with 50%ethanol by using Soxhlet Apparatus. The extractswere concentrated and dried at 60^0c as semisolid and kept at 4^0c for further studies (Singh *et al*., 2013). Fresh solution of *A. paniculata* was prepared at the time of experimentation.

3.3. Experimental design

Three to five months old albino Wistar rats of either sex of approximately 130 – 150 gm weight were randomly divided into three groups, having eight rats in each groups.

I: Control group : Rats received intraperitoneal injection of distilled water (0.5 ml) for eight weeks.

II: Induced group : Rats which were given intraperitoneal injection of freshly prepared thioacetamide solution at a dose of 200 mg/kg body weight three times a week for eight weeks.

III: Treated group: Induced Rats which were given ethanolic extract of *A. paniculata* orally at a dose of 250 mg/kg body weight three times a week for eight weeks.

3.4. Blood sample collection

After experimentation, experimental rats were fasted for 12 hrs. and sacrificed by cervical dislocation. Blood sample was directly collected from the heart i. e. left ventricle and was allowed to clot at room temperature. After clotting of blood the supernant was collected carefully and was centrifused at 3000 rpm for about 15 minutes and serum obtained at the top of tube was collected for further experiment.

3.5. Biochemical methods

i) Estimation of creatinine by Jaffe's alkaline picrate method.

ii) Estimation of blood urea nitrogen, serum sodium and potassium was estimated by using commercial Kits.

III. RESULTS

The results obtained in the present investigation are shown in the table and are illustrated graphically in the figs.1, 2, 3 and 4. The level of serum creatinine, BUN, serum sodium and potassium in control group was0.7582± 0.1114mg/dI, 46.4±2.0736mg/dI,134.4±1.356mmol/L and 3.328± 0.258mmol/Lrespectively which was found increased moderately in induced group upto1.702±0.1663mg/dI, 96.6±1.1402mg/dI, 155±2mmol/L and 5.044± 0.1514mmol/L respectively.

The elevated level of serum creatinine, BUN, serum sodium and potassium in TAA induced rat was found moderately significant decreased with administration of ethanolic extract of *A. paniculata* where the values were 1.108± 0.2146mg/dI, 64±3.6742mg/dI, 146.6±1.35 mmol/L and 3.854± 0.1510mmol/L respectively at a dose of 250 mg/kg of b. w.

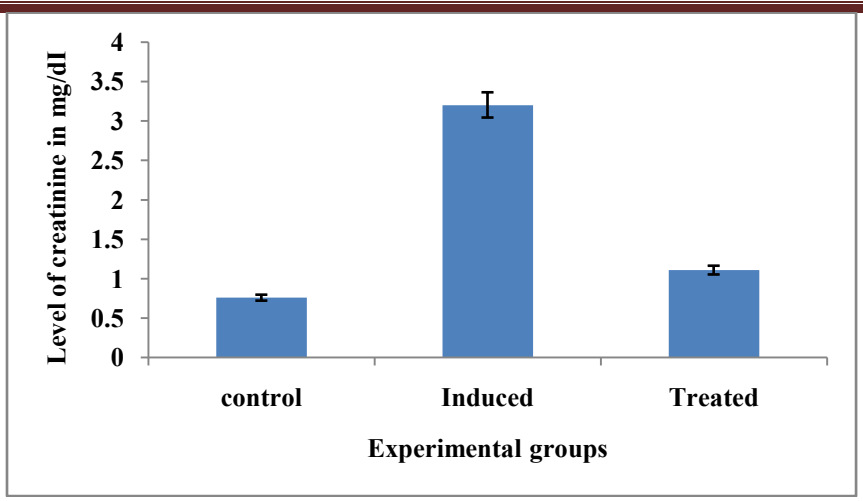

Figure 1 : showing level of serum creatinine in different groups of rat

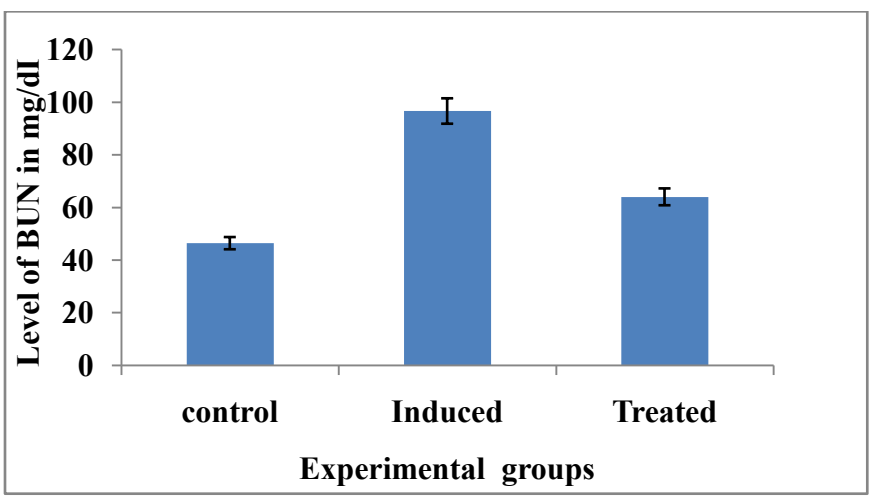

Figure 2 : showing level of BUN in different groups of rat

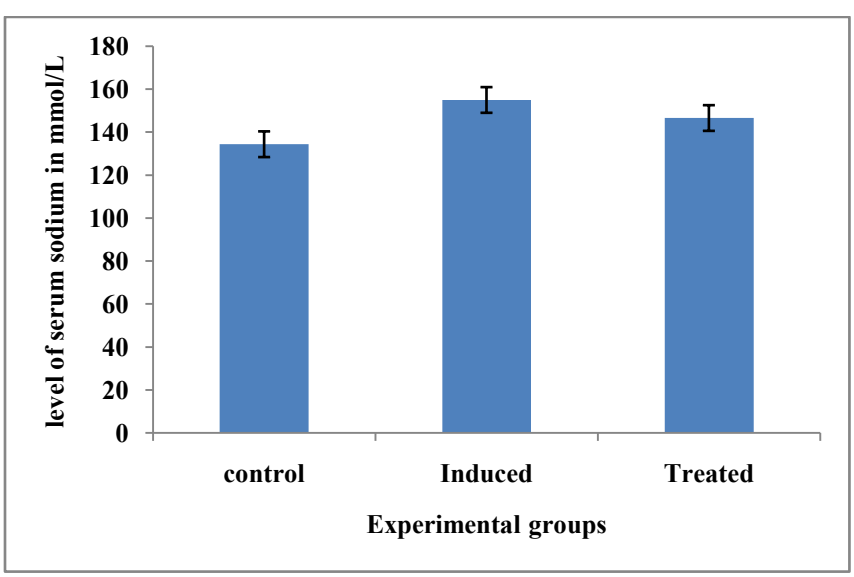

Figure 3 : showing level of serum sodium in different groups of rat

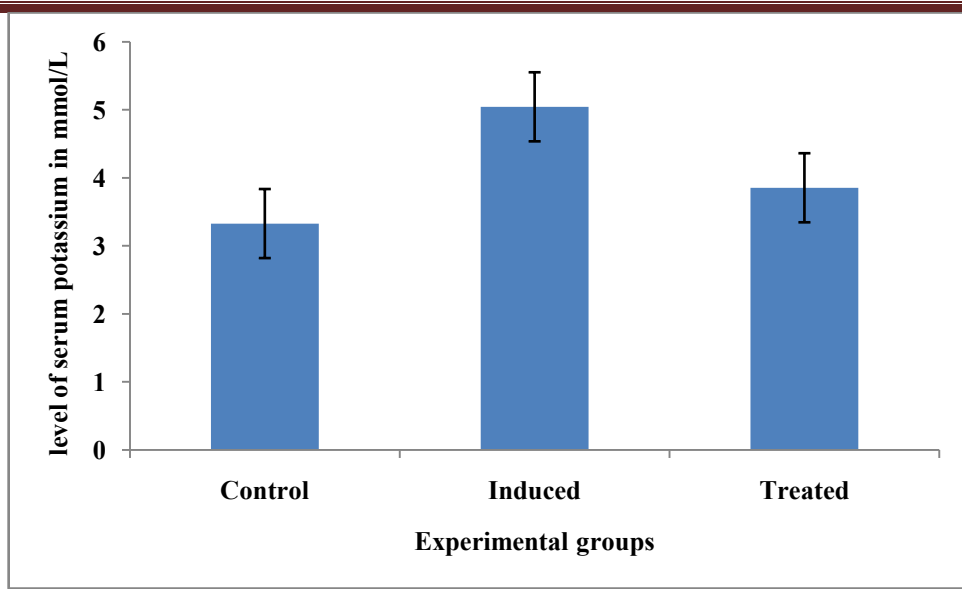

Figure 4 : showing level of serum potassium in different groups of rat

Table : Showing the effect of ethanolic extract of *A. paniculata* on the level of creatinine, BUN, Na and K in different groups of rat.

Parameter	Animal Groups		
	Control	Induced	Treated
Creatinine (mg/dl)	0.7582± 0.1114	1.702±0.1663**	1.108± 0.2146**
BUN (mg/dl)	46.4±2.0736	96.6±1.1402**	64±3.6742**
Na (mmol/L)	134.4±1.356	155±2**	146.6±1.35**
K (mmol/L)	3.328± 0.258	5.044± 0.1514**	3.854± 0.1510**

Each value is the mean of 8 individual determinations, ± indicates SD
*** P < 0.001 highly significant, ** P < 0.01moderately significant, * P < 0.05 significant, ^{NS} P>0.05 Non significant

IV. DISCUSSION

In the present study, nephroprotective effect of ethanolic extract of *A. paniculata* was tested against thioacetamide induced nephrotoxicity in white rats. For the measurement of renal function condition serum urea and creatinine are regarded as dependable markers (Adelman *et al.*, 1981). Thioacetamide is mostly used as a hepatotoxin to recognized animal models of acute and chronic liver injury (Peeing et al. 1993; Zimmermann et al. 1987). Thioacetamide effects not only in liver also found structural and functional changes in the kidney (Barker and Smuckler, 1974), thymus (Barker and Smuckler, 1973), spleen (Al-Bader et al., 2000) and the intestine (Ortega et al. 1997).TAA is a weak carcinogen which affects on liver and kidney (Kleinfeld, 1957; Bruck et al., 2007; Hasegawa et al., 2007; Rekha et al., 2008) and it causes histological changes in proximal convoluted tubule (Baker and Smuckler, 1974) it increases size of the cortical kidneynuclei and nucleoliand reduces activity of glucose-6-phosphatase activity of the nuclear envelope (Inciate and Gonzalez-Mujica et al.,1993).TAA is also a most important nephrotoxinfor the reason that of rapid elimination and collective injury when it is given intermittently, most probably by free radical mediated lipid (Begum et al., 2011; Dashti et al., 1997).

The Creatinine, urea and electrolytes are the importantresponsive biochemical markers working in the analysis of kidney damage, the creatinine and BUN are excreted through kidney
while electrolytes are reabsorb and excreted in the tubule (). As per Anwar *et al.* (1999) increased serum level of urea and creatinine used as a index of nephrotoxicity. The serum level of creatinine, BUN and uric acid are helpful tools in diagnosis as they choice any conflict to the renal system early sufficient to allow for projection and possible remedies.Bheeman et al. (2013) stated that measurement of ion concentrations are used as potential biomarkers of chemical exposure and effects. Sirag (2007) reported elevations of serum creatinine, urea and potassium in TAA intoxicated albino rats. Begum et al. (2011) reported that raised level of urea and creatinine in TAA intoxicated Wistar rats. According to them elevated level of urea and

creatinine shows insufficiency of renal functions. Increased level of serum creatinine, BUN and uric acid in mice have also been reported chronically intoxicated with TAA by (Al-Attar, 2012).Histopathologically renal tissue shows congestion of the glomeruli, focal mesengial cell proliferation, increased deposition of collagen in renal medulla, fibrin in cortex, distrupted and swollen cells ofconvoluted tubules and lobulated atrophied glomeruli, tubular epithelial cell necrosis and inflammatory cell infiltration (Al-Bader et al., 1999; Mahmoud, 2006; Kadir et al., 2013). In the present investigation intoxication of TAA shows moderately significantly increased level of serum creatinine, BUN, sodium and potassium in albino rats when compared with control rats. Significantly elevated level of blood urea and serum creatinine in TAA intoxicated group was reported by Kadir, *et al.*(2013).In the present study significant elevation observed in the level serum creatinine, BUN,sodium and potassium might be due to formation of congestion of the glomeruli and swollen cells of convoluted tubules in the kidney in TAA intoxicated rats.

In the previous studies carried out by most of the investigators it is mentioned that elevated serum levels of creatinine, urea, alkaline phosphatase, BUN, sodium and potassium in rats intoxicated with certain toxic material was found decreased after the treatment with extracts of certain plant material. Kadir*et al*. (2013) have reported that administration of ethanolic extract of *Vitexnegundo*against thioacetamide treated rats showed a significant decrease in the levels of blood urea and serum creatinine when compared with thioacetamide treated rats. According to Al-Attar *et al.* (2015) insignificant decline in the level of TAA intoxicated mice serum creatinine, BUN and uric acid in male mice treated with olive and juniper leaves extract. Sarvankumar*et al.* (2011) revealed that significant reduced level of SGOT, SGPT, ALP, total protein, urea, creatinine and bilirubin in experimental rats after treated with methanolic extract of *Vitexnegundo* barkin paracetamol treated rats. The decline in the level of blood urea and serum creatinine in gentamicin intoxicated rats after treated with administration of ethanolic extract of *Tephrosiapurpurea*in rats was reported by Jain *et al*. (2013). The decrease in the level of serum creatinine BUN, sodium and serum potassium observed in the present investigation after the oral administration of ethanolic extract of *A. paniculata* are in accordance with the papers mentioned by earlier workers in such type of studies. The ethanolic extract of whole plant of *A. paniculata* might have contains the flavonides and alkaloids which might have shows the hepatoprotective activity.

V. CONCLUSION

The results obtained in the present investigation clearly indicated the nephroprotective effect of ethanolic extract of *A. paniculata* against thioacetamide induced nephrotoxicity in white rat.

VI. ACKNOWLEDGEMENT

The author is thankful to Principal and Head, P.G. Dept. of Zoology, S. G. M. College, Karad, for provided that the necessary facilities during present work.

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EFFECT OF *WITHANIA SOMNIFERA* ON GLYCOPROTEINS CONTENT IN EXOCRINE GLANDS OF AGED MICE(*MUS MUSCULUS*)

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ABSTRACT: *Varieties of antioxidants are reported by researchers to reduce the adverse effect of ageing. The present investigation was undertaken to study the effect of Withania somnifera on glycoproteins content in exocrine glands of aged mice. The experimental animals were divided in to four groups 1) Young mice 1-2 months 2) Adult mice 5-6 months 3) Old mice 16-18 months 4) old mice + Withania somnifera .The exocrine glands namely submandibular glands, sublingual glands, parotid glands and exocrine pancreas were taken for the study. The leaf extract of Withania somnifera was given subcutaneously to the old mice for 20 days (40mg/Kg body weight). In old mice of both sexes, the Glycoproteins content was significantly increased as compared to young and adult male and female mice, but when, the old mice were fed with dose of Withania somnifera (40gm/kg body weight) per day for 20 days. It was observed that the Glycoproteins content was decreased as compared to old mice. Therefore, old groups suggesting that Withania somnifera haveanti-agingproperties.*

Keywords: *Exocrine glands, Withania somnifera, Glycoproteins.*

I. Introduction

Salivary glands are readily accessible and well characterized. Therefore they are useful tools for the study of the normal ageing process and the impact of stress on organ reserve and secretary functions observed by Baum B.J. et al 1992. The main functions of salivary glands are to secrete saliva and also secrete enzymes, hormones pharmacologically active components like amylase, peroxidase reported by several researchers. (Young J.A. and Van Lennep, 1978; Barka, T.1980; and Spone, M. B. et al 1982).Several researchers studied that the Glycoproteins are protein having covalently bound carbohydrates and they are found in all living organisms in both soluble and insoluble form with diverse functions and properties.

The aging process is one of the serious problems of the modern world. Due to various medicines against terminal diseases like cancer, diabetes, atherosclerosis, rheumatism and various infectious diseases, life is saved but ageing process is not stopped. Modern medicines are unable to solve the problems of old age and disability (Ship *et al.,* 2002). Salivary glands are affected due these medicines and also due to the old age. The root cause is free radicals formed during aging, due to stress or toxicity of various medicines. Free radicals have been implicated in etiology of several human diseases as well as aging (Halliwell & Gutteridge, 1997). In old age free radicals are not removed efficiently by defense mechanism of the cell which includes Super Oxide Dismutase (SOD), Catalase CAT and Glutathion Peroxidase (GPx) and some other antioxidants. There is a long list of antioxidants, suggested by various scientists and flavonoids are supposed to be very good antioxidants extracted from various plants. Withania somnifera is an amazing and popularly used ayurvedic plant commonly called as 'Indian ginseng' (Bhatnagar et al., 2005). It acts as anti-stress, adaptogenic agent as well as increases life span and delay ageing (Satyavati, 1995). Pillai et al 1998 observed that the sulphated glycoproteins rich in salivary glands which includes hexoses, fucose and sialic acids. Shah M.H. et al (2008) reported that the increased metabolism of sialoglycoproteins and sialoglycolipids are observed, which is considered a cause of elevated serum total and lipid bound sialic acid in blood serum of cancer patients.Bhattacharya S.K.et al (1997) showed that the glycowithanolide is bioactive chemical principle of Withania somnifera and has beneficial effect in treatment of stress observed by Grandhi et al (1994). The present investigation the study used for the assessment of antiageing activity of Withania somnifera in aged mice.

In present study glycowithanolides extracted from Withania somnifera leaves was tested to find out its effect on fucose content in salivary glands of mice during aging. Salivary glands play important role in growth, differentiation and development (Bodre & Pillai, 2007; Walvekar & Pillai, 2008). Several biologically active polypeptides such as Epidermal Growth Factor (EGF), Nerve Growth Factor (NGF), and Transforming Growth Factor (TGF) etc. are secreted by salivary glands (Sporn et al., 1982). During old age salivary glands undergo changes in morphology (Kim and Allen 1993), histology (Scott et al., 1986), biochemistry (Denny et al., 1991a&b; Mahay et al., 2004) and ultrastructure (Bogart, 1970). Several studies reported diminished functions of salivary glands leads to various old age related diseases such as xerostomia, dental caries,

Sjogren's syndrome, periodontal disease etc (Olver et al., 2006). There is a close relationship between oral and systemic health (de- Almeida et al., 2008). Fucose is deoxyhexose sugar and is found in N-linked glycanes on the mammalian, insect and plant cell surface. It is required for optimal functions of cell to cell communication. It is a powerful immune modulator. It has significant role in slowing the growth of cancer cells. Its deficiency is accompanied by a complex set of phenotypes both in human and mice. Fucosylated glycanss have been implicated in the rheumatoid arthritis patient (Flogel et al., 1998; Gornik et al., 1999) in cystic fibrosis (Scanlin & Glick, 1999).

Fucose deficiency in animal causes a large number of phenotypic consequences, underscores the crucial role of fucosylated glycanes to many physiological and developmental processes (Baker & Lowe, 2003). For fucosylation fucose is obtained from GDP- fucose. GDP-fucose is synthesized by de- Novo and salvage pathway (Tonetti et al., 1998). In salvage path way free fucose required for GDP-fucose synthesis is derived from extracellular of lysosomal fucoseor lysosomal catabolism of glycoproteins and glycolipids. GDP-fucose thus synthesized is then transported into lumen of the Golgy apparatus for fucosylation (Baker & Lowe, 2003). Thus lysosomes and Golgi apparatus play important role in fucosylation. This shows that fucosylated glycoproteins play important role in salivary glands which are biomarkers of aging. It is essential to study the fucose in salivary glands and prevention of its loss during aging and stress. In the present study WSG extracted from Withania somnifera was used to prevent the loss of fucose from aged and stressed salivary glands.

II. MATERIALS AND METHODS

16 to18 months old male and female mice (Mus musculus) weighing were selected for present investigation. They were obtained from Hindustan Antibiotices, Pune. They supplied with Amrut mice feed (Pranav Agro Industries Ltd. Sangli) and clean water ad libitum. Animals were reared in the air conditioned departmental animal house.

Group A: Young male and female mice (1to 2 months) control group
Group B: Adult male and female mice (5 to 6 months) control group
Group C: Old male and female mice 16 to 18 months) control group
Group D: Old male and female mice + Withania somnifera induced group: Mice were treated with the dose of Withania somnifera (40mg/kg body weight) per day for 20 days. All treatments were given at 9.00 am.

After completion of treatment, animals were sacrificed by cervical dislocation between 9.00 am to 12.00 pm in noon exocrine glands were pulled, weighed, homogenized, and centrifuged at 5000 rpm for 10 minutes at 10 0C temperature to prepare sample and used for the estimation of Sialic acid by Warren et al. (1959) and Fucose by Dische and Shettles (1948) method.

Preparation of plant extract: Fresh leaves of Withania somnifera were shade dried, crushed and chloroform extract was prepared as described by Bhattacharya et al. The aqueous concentrate of Withania somnifera leaves was exhaustively extracted with chloroform to remove fatty materials and free withanolides. The later was determined with the help of GCMS. Withania extract was freely soluble in water and saline. Plant extract was dissolved in sterile water and was given to the experimental mice subcutaneously (40 mg/kg body weight).

III. RESULTS AND DISCUSSION

1) Effect of Withania somnifera on the Sialic acid content (µg/mg tissue) in Exocrine gland of aged male mice. (Table No.:1)

In young and adult male mice, the sialic acid content in sublingual gland was 0.00039 ± 3.3834µg/mg and 0.00050 ± 4.1726 µg/mg respectively. The sialic acid content in sublingual gland showed significant decrease ($p<0.001$) in the old male which was administrated with Withania somnifera as compared with the sialic acid content in sublingual gland of male mice.

In young male mice, the sialic acid content in submandibular gland was 0.0001489 ± 2.1890 µg/mg, while in adult male mice, the sialic acid content in submandibular gland was 0.000225 ± 3.8183 µg/mg. there was significant increase in the sialic acid content in submandibular gland of adult male mice as compared with the young male mice. There was significant decrease ($p<0.001$) in the sialic acid content in submandibular gland of old male mice as compared to adult male mice. But the significant decrease ($p<0.001$) was observed in the sialic acid content in submandibular gland of old mice which was treated with Withania somnifera for 20 days The sialic acid content in parotid gland of young male mice was 0.00016 ± 2.4883 µg/mg. The sialic acid content in parotid gland of adult male mice showed significant

increase in its sialic acid content (p<0.001). The sialic acid content in parotid gland of old male mice was 0.00024 ± 2.1294 µg/mg. the old male mice was administrated with Withania somnifera for 20 days. After the treatment with Withania somnifera the sialic acid content in parotid gland of old male mice was increased significantly (p<0.001) as compared to the parotid gland of old male mice.The sialic acid content in pancreas of young male mice was 0.00023 ± 1.9075µg/mg. In adult male mice, the sialic acid content in pancreas was 0.00033 ± 2.7767µg/mg. As compared to young male mice, there was significant increase (p<0.001) in the sialic acid content in pancreas of adult male mice. The pancreas of old male mice was 0.00034 ± 2.7249 µg/mg which showed significant increase (p<0.001) in the sialic acid content as compared to adult male mice. When the old male mice was orally administrated with Withania somnifera for 20 days, it was found that there was significant decrease in the sialic acid content in pancreas as compared with the sialic acid content in pancreas of old male mice.

2) Effect of Withania somnifera on the Sialic acid content (µg/mg tissue) in Exocrine gland of aged female mice. (Table No.:2)

The sialic acid content in sublingual gland in female mice is shown in table No 2. The sialic acid content in sublingual gland (p<0.001) was significantly increased in the adult female mice as compared to young female mice. There was also significant decrease the sialic acid content in sublingual gland of old female mice which was administrated with Withania somnifera for 20 days as compared to the sialic acid content in sublingual gland of old female mice.

The sialic acid content in submandibular gland of young and adult female mice was 0.0001270 ± 1.5256 µg/mg and 0.0002032 ± 2.3453 µg/mg respectively. In old female mice, the sialic acid content in submandibular gland was 0.00022 ± 5.7534 µg/mg. After the treatment with Withania somnifera the sialic acid content in submandibular gland of old female mice showed significant decrease (p<0.001) as compared to the old female mice. In young female mice the sialic acid content in parotid gland was 0.00013 ± 1.6842µg/mg. There was significance increase (p<0.001) in the sialic acid content in parotid gland of adult female mice. In old female mice, there was significant increase (p<0.001) in the sialic acid content in parotid gland. After the treatment of Withania somnifera for 20 days, there was significant decrease (p<0.001) in the sialic acid content in parotid gland as compared with the sialic acid content in parotid gland of old female mice. In young and adult female mice, the sialic acid content in pancreas was 0.00022 ± 2.2060 µg/mg and 0.00029 ± 1.9298 µg/mg respectively. The sialic acid content in pancreas of old female mice was 0.00032 ± 3.6588 µg/mg there was significant increase (p<0.001) in the sialic acid content in pancreas of old female mice. But after the treatment of Withania somnifera in old female mice, the sialic acid content was reduced . There was significant decrease in the sialic acid content in pancreas of old female mice.

3) Effect of Withania somnifera on the Fucose content (µg/mg tissue) in Exocrine gland of aged male mice. (Table No.:3)

In young male mice, the fucose content in sublingual gland was 0.8140 ± 0.0207 µg/mg, while in adult male mice, the fucose content in sublingual gland was 1.4500 ± 0.0224 µg/mg. there was significant increase in the fucose content in sublingual gland of adult male mice as compared with the young male mice. There was significant decrease (p<0.001) in the fucose content in sublingual gland of old male mice as compared to adult male mice. But the significant increase (p<0.001) was observed in the fucose content in sublingual gland of old mice which was treated with Withania somnifera for 20 days. The fucose content in submandibular gland of young male mice was 0.7040 ± 0.0207 µg/mg. In adult male mice, the fucoe content in submandibular gland was 1.2260 ± 0.207 µg/mg. As compared to young male mice, there was significant increase (p<0.001) in the fucose content in submandibular gland of adult male mice. The fucose content in submandibular gland of old male mice was 0.4080 ± 0.0303 µg/mg which showed significant decrease (p<0.001) in the fucose content in submandibular gland as compared to adult male mice. When the old male mice was administrated with Withania somnifera for 20 days, it was found that there was significant increase in the fucose content in submandibular gland (0.9080 ± 0.2770 µg/mg) as compared to the fucose content in submandibular gland of old male mice.

The fucose content in parotid glands of young male mice was 0.6260 ± 0.0241 µg/mg. The fucose content in parotid glands of adult male mice observed significant increase in its protein content in parotid glands (p<0.001). The fucose content in parotid glands of old male mice was 0.3340 ± 0.0241 µg/mg. When old/ aged male mice was treated with the dose of Withania somnifera for 20 days. After the treatment of Withania somnifera the fucose content in parotid glands of old male mice was increased significantly (p<0.001) as compared to the parotid gland of old male mice.

The fucose content in pancreas of young and adult male mice was 0.8440 ± 0.0385 µg/mg and 1.3000 ± 0.0158 µg/mg respectively. In old male mice, the fucose content in pancreas showed the 0.4660 ±

0.0241 µg/mg which was slightly reduced ($p<0.001$) as compared to the fucose content in pancreas in adult male mice. When the old male mice was treated with the dose of Withania somnifera for 20 days .After the treatment with Withania somnifera the fucose content in pancreas of old male mice was significant increase ($p<0.001$) as compared to the fucose content in pancreas of old male mice.

4) Effect of Withania somnifera on the Fucose content (µg/mg tissue) in Exocrine gland of aged female mice. (Table No.:4)

The fucose content in sublingual gland of young and adult female mice was 0.7160 ± 0.0207 µg/mg and 1.3200 ± 0.0316 µg/mg respectively. In old female mice, the fucose content in sublingual gland was 0.3210 ± 0.0314 µg/mg. After the treatment with Withania somnifera the fucose content in sublingual gland of old female mice showed significant increase ($p<0.001$) as compared to the old female mice. In young and adult female mice, the fucose content in submandibular gland was 0.6400 ± 0.0158 µg/mg and 1.1640 ± 0.0305 µg/mg respectively. The fucose content in submandibular gland of old female mice was 0.3540 ± 0.0230 µg/mg. there was significant decrease ($p<0.001$) in the fucose content in submandibular gland of old female mice. But after the treatment of Withania somnifera in old female mice, the fucose content was regained (0.8540 ± 0.0241 µg/mg). There was significant increase in the fucose content in submandibular gland of old female mice. The fucose content in parotid glands of young female mice was 0.5800 ± 0.0316 µg/mg. There was significant increased ($p<0.001$) in the fucose content in parotid glands (0.9200 ± 0.0316 µg/mg) of adult female mice. In old female mice, there was significant decrease ($p<0.001$) in the fucose content in parotid glands (0.3280 ± 0.0259 µg/mg). But after the treatment of Withania somnifera for 20 days, there was significant increase in the fucose content in parotid glands as compared with the fucose content in parotid glands of old female mice. The fucose content in pancreas of young and adult female mice was 0.7740 ± 0.0230 µg/mg and 1.1460 ± 0.0385 µg/mg respectively. In old female mice, the fucose content in pancreas of was 0.3780 ± 0.0192 µg/mg. After the treatment of Withania somnifera for 20 days, fucose content in pancreas of old female mice showed significantly increase ($p<0.001$) as compared to the old male mice. It was observed that the fucose content in pancreas of female mice was less than the male mice.

IV. DISCUSSION

In old mice of both sexes, the sialic acid content was significantly increased as compared to young and adult male and female mice, but when, the old mice were fed with dose of *Withania somnifera* (40gm/kg body weight) per day for 20 days. It was observed that the sialic acid content was decreased as compared to old mice of both sexes (16th-18th months).

In stressed condition, salivary glands sialoglycoproteins and also o-acetylated sialic acids are increased and Baum, B.J. (1987) and Rajakumar G and Scarpace P.S.(1991) showed that the increase in sialic acid content in stressed condition mice might be due to accumulation of glycoproteins in acini, due to old age of inhibition of protein secretions. Emmanouil-Nikoloussi et al (1992) reported that during ageing the submandibular glands of rat, the accumulation of neutral and acid glycoproteins in acinar cells. Gokman et al (2000) studied that increased accumulation of glycoproteins content may also due to increase in lipid peroxidation and secretion of sialic acid from cell to cell membrane surface may be partly responsible for increased sialic acid concentration. Beatty P et al. (2001) reported that increased of FSA may be stimulated by the higher release of sialic acid from surface pancreatic glycoconjugates as well as from other hydrolyzed structure. Hedlund M. et al (2008) reported that the increase in serum concentration of sialic acid may be explained by the intense proliferation and degradation of tumor cells.

Kalmade et al (2007) reported in prostate gland, increase in o-acetylated sialic acid in D-galactose stressed mice, and increase in salivary glands studied by Sonavane et al (2007). Tomake et al (2008) showed that increase in sialic acid content in naturally aged old male rat's salivary glands.

In old mice of both sexes, the Fucose content was significantly decreased as compared to young and adult male and female mice, but when, the old mice were fed with dose of *Withania somnifera* (40gm/kg body weight) per day for 20 days. It was observed that the fucose content was significantly increased as compared to old mice of both sexes (16th-18th months).

The reduction in the fucose content of salivary glands may be due to the reduction in glycoprotein synthesis. The progressive decline in the rate of protein synthesis with age in the salivary glands was described in rat (Kuatt & Baum, 1981, Baum *et al.,* 1983, Rattan, 1996) in mice (Mote *et al.,* 2009). This decline in protein synthesis is due to free radicals induced structural damage in salivary glands cells (Scott,1977a; Azevedo *et al.,* 2005; Mote *et al.,* 2010). D-galactose induces oxidative stress followed by AGEs (Song *et al.,* 1999; Deshmukh *et al.,* 2006). The changes observed in salivary glands of D-galactose stressed

mice such as reduction in total proteins (Mote *et al.,* 2009), structural damage (Gresik, 2005) etc are similar to the changes observed in naturally aged animals (Brian *et al.,* 1981, Kim & Allen, 1993; Azevedo *et al.,* 2005). Similarly the increase in lipid peroxidation in brain (Lee *et al.,* 1997), in mitochondrial fraction of brain (Vora *et al.,* 2009), alterations in lysosomal enzymes (Vora *et al.,* 2005; Pillai *et al.,* 2003) were observed in D-galactose stressed mice. impaired due to damage to these cell organelles. Damage to the cell organelles during aging was reported by Sashima (1986), Ashour (1998) in rat salivary glands. When D-galactose stressed adult and old mice were treated with CPH and WSG there was recovery in fucose content of submandibular and sublingual glands of both protective and curative groups of adult. The recovery was not up to the normal level in old. These antioxidants may help in removal of free radicals. CPH possess OH-radical scavenging capacity (Zs-Nagy, 1989), which can help to protect the cellular damage. WSG is a powerful natural antioxidant described by several researchers (Bhattacharya *et al.,* 1997; Naidu *et al.,* 2006; Kumar *et al.,* 2005; Harikrishna *et al.,* 2008; Rajasankar *et al.,* 2009). The antioxidant potential of *Withania somnifera* inhibit ROS induced lipid peroxidation (Gupta *et al.,* 2003; Kumar *et al.,* 2006; Palanyandi , 2006)which may prevent damage of Golgi, ER and other cell organelles and they remain intact to carry out cellular function. WSG increases cell's antioxidant enzymes i.e. SOD, CAT and GPx in Wistar rats (Gupta *et al.,* 2003; Naidu & Singh, 2006) and prevent free radical mediated cellular damage. Though with CPH and WSG there is recovery of fucose content in salivary glands both in D-galactose stressed adult and old mice it is more significant in WSG treatment.Though CPH and WSG are capable of recovery of fucose content and the structure of salivary glands, this is remarkable in the adult mice treated with D galactose but in old mice (16 to 18 month old) this recovery is not like that of adult. This shows that during normal aging there may be permanent loss of certain cellular structures due to free radicals which are not removed or regenerated afterwards. This shows that WSG can be useful in treatment of alterations in salivary glands due to certain diseases like xerostomia, cancer or other medicines. But physiology of old salivary glands can't be changed up to satisfaction.

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SURVEY OF WADERS IN THE CATCHMENT AREA OF NAGESHWARWADI TANK TQ. AUNDHA (NAG.) DIST. HINGOLI (M.S.) INDIA

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ABSTRACT: *Nageshwarwadi tank is ½ km. away from Nageshwarwadi village near about 5 km. from Aundha (Nag.) taluka, Dist. Hingoli. The tank is constructed for irrigation project govern by Govt. of Maharashtra irrigation department. The water spread area of the tank is 65 hectore. Nageshwarwadi tank provides feeding and roosting grounds for migratory and resident waders. The study was conducted during June 2016 to may 2017. In present study total 11 species of waders were identified belonging to 4 different families of Charadriidae, Glariolidae, Recurvirostridae and Scolopacidae.*

Keywords: *Waders, migratory, Nageshwarwadi tank, Aundha (Nag.)*

I. Introduction

Wetlands are vital components of our ecosystem. The waders are recognized as one of the major group of water birds these are habituated only in marshy and muddy parts of aquatic bodies (Kumbhar and Ghorpade 2015). Waders are an important component of the mangrove ecosystem and their occurrence and distribution help to understand the overall picture of the wetland habitat (Chaudhari-Pachpande and Pujaver 2016). Charadriiformes is a divers order of small to medium- large birds (Manikannan et. al. 2012).The order of waders are charadriiformes which included in different families as Burhinidae, Charadriidae, Glareoldae, Jacanidae, Recurvirostridae, Rostratulidae and Sclopacidae. Some distinguishing physical characteristics of the waders are long, thin legs, long and slender forefingers, long bill, neck, plumage, well developed and more flexible ligament and tendons of knee region. Wetland ecosystem harbors many migratory and common resident wading birds as well as they provide food for birds in the form of some aquatic plants, fishes, invertebrates, larvae and insects, etc. Waders have been seen wading through the shallow waters and occasionally probing along dry margins of the wetland (Mishra et.al.2016).

The present work is focused on Survey of waders in the catchment area of Nageshwarwadi tank Tq. Aundha (Nag.) Dist. Hingoli because of there is no any studies about waders.

II. MATERIALS AND METHODS

The survey of waders in the catchment area of the Nageshwarwadi tank is situated ½ km. from Nageshwarwadi village and 5 km. away from Aundha (Nagnath). The shore of the tank is muddy. The soil is a silty clay type which is present less amount of sand. The shore of the tank shows a phytoplankton, zooplankton and insects. The present study of waders was conducted for the period of June 2016 to may 2017. The survey of wader was carried out by monthly visits to the tank in the morning (7.00 to 9.30 Am.) and evening (4.00 to 6.30 Pm.). The study was carried out by using binocular 5x420IS (42 X).The waders are identified by using the book of Indian birds by Salim Ali BNHS 2012, Birds of Maharashtra Ela foundation 2011.

III. RESULTS AND DISCUSSION

In study area total 11 waders were recorded. All recorded waders are included in the Charadriiformes belonging to 4 different families viz. Charadriidae, Glariolidae, Recurvirostridae and Scolopacidae. The family Charadriidae 5 sp. was recorded out of these 3 was winter migratory (Common ringed plover, Greater sand plover and Grey plover) and 2 resident (Little ringed plover and Red wattled lapwing), In Glareolidae family only 1 small prantincole species is recorded, in Recurvirostridae family only 1 winter migratory Black winged stilt is recorded and Scolopacidae 4 species was recorded and all species was winter migratory which included Broad billed sandpiper, Curlew sandpiper, Little stint and Wood sandpiper.

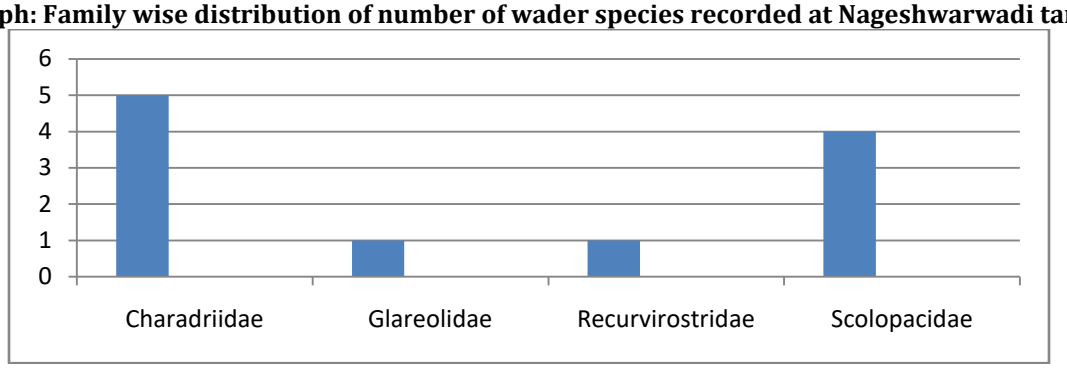
The work on wader is done by A. C. Kumbhar and B. N. Ghorpade (2015) recorded 17 waders in the catchment area of Ujani dam, near Bhigvan, P.V. Darekar *et al.* (2017) recorded 17 waders in the catchment area of Sangvikati percolation tank Tal. Tuljapur dist. Osmanabad, Mishra *et.al.,* (2016) recorded 28 waders in a natural wetland Bakhira tal. dist. Santkabirnagar U.P. India.

Table: List of waders recorded at Nageshwarwadi tank.

Sr. No.	Family	Sr. No	Common Name	Scientific Name	Status
1	Charadriidae	1	Common ringed plover	*Charadrius hiaticula*	WM
		2	Greater sand plover	*Charadrius leschenaultia*	WM
		3	Grey plover	*Pluvialis squatarola*	WM
		4	Little ringed plover	*Charadrius dubius*	R
		5	Red wattled lapwing	*Vanellus indicus*	R
2	Glareolidae	6	Small prantincole	*Glariola lacteal*	R
3	Recurvirostridae	7	Black winged stilt	*Himantopus himantopus*	WM
4	Scolopacidae	8	Broad billed sandpiper	*Limicola Falcinellus*	WM
		9	Curlew sandpiper	*Calidris ferruginea*	WM
		10	Little stint	*Calidris minuta*	WM
		11	Wood sandpiper	*Tringa glareola*	WM

R=Resident, WM= Winter Migrant.

Graph: Family wise distribution of number of wader species recorded at Nageshwarwadi tank.



IV. CONCLUSION

A total of 11 species of waders belonging to 4 different families have been recorded at Nageshwarwadi tank during the period from June 2016 to may 2017. The winter migratory waders were larger than the resident wader. It can be concluded that the large varitey of birds attracted towards the tank due to large variety of food were available at the tank. The present survey will be helpful for the future study.

V. ACKNOWLEDGEMENT

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TRIACYLGLYCEROL ESTER HYDROLASE ACTIVITY DURING MOTH LONGEVITY OF INSECT, *EARIAS VITTELLA* (FABRICIUS)

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ABSTRACT: *Triacylglycerol ester hydrolase activity during moth longevity of insect, Earias vittella (F.) has been studied. Triacylglycerol ester hydrolase activity was maximum at pH 7.8 in male moth and pH 7.7 in female moth. The K_m value was noted to be 0.13 × 10^{-2}mM and 0.20× 10^{-2} mM respectively for male and female moth. The specific activity of triacylglycerol ester hydrolase activity revealed 25.075 and 24.274 µmol FFA /mg protein /25 minutes respectively of 7-day male moth and of 8-day female moth of insect, E. vittella. The physiological role of lipase during longevity of insect moth, E. vittella has been discussed.*

Keywords: *Triacylglycerol ester hydrolase activity (EC 3.1.1.3), male and female adult longevity, E. vittella.*

I. Introduction

Earias vittella is serious pest of okra. It attacks growing points and feed mostly inside the flowers and fruits. The economic injury level of *E. vittella* on okra is reported to be 5.3% damage (Krishnaian *et al*., 1978). The insect fat body which combines many of the properties and functions like storage of lipids (Law and Well, 1989). Lipases are ubiquitous enzymes of considerable physiological significance and industrial potential. Lipases catalyse the hydrolysis of triacylglycerol to glycerol and free fatty acids (Martinelle *et al*., 1995). Okra has great diversity in different parts of the country particularly in the western lowlands regions (Schippers, 2000). Many studies have been carried out on the triacylglycerol lipase activity of various insects (Tembhare and Muthal, 1992; Smith *et al*., 1994; Uscian *et al.,* 1995; Rana *et al*., 1997; Schippers, 2000; Auerswald and Gade, 2006; Grillo, *et al* 2007, Saadati and Mirzaei, 2016 and Wang*et al.* (2016). The information on triacylglycerol ester hydrolase activity in male and female adult development of *E. vittella* is rather scanty. In present study, an attempt has been made to estimate triacylglycerol ester hydrolase activity during mothlongevity of insect, *E. vittella* which mainly concerned with liberation of energy for active life of moth.

II. MATERIALS AND METHODS

The rearing of *E. vittella* was maintained in the laboratory on natural food of okra fruits (Roqaya, 2000). Male moths from 1-day to 16-days and female moths from 1-day to 18-day were used for study. Partial purification of triacylglycerol lipase was attempted by ammonium sulphate precipitation method (Dawson *et al.,* 1969). Lipase assay include 0.25 ml of substrate, 0.25ml partially purified lipase enzyme and 1 ml of phosphate buffer pH 7.8 in total volume of 1.5 ml. The absorbance was read at 540 nm(Hayase and Tappel, 1970). Protein estimation method included 0.5 ml partially purified enzyme, 4.5 ml of reagent I mixed well and allowed to stand for 10 minutes of incubation at room temperature. Immediately, 0.5 ml reagent II was added rapidly performing the total volume of 5.5 ml. Reagent I contained 2 % Na_2CO_3 in 0.1N NaOH, 1 % sodium tartrate in distilled water and 0.5 % $CuSO_4$ in distilled water. Reagent II included 1 part of Folin and Ciocateu's reagent (phenol reagent) [2N] and 1 part of water. After 30 minutes of incubation reading was taken calorimetrically at 750 nm. Reagent I and Reagent II were prepared freshly just before experiment(Lowry *et al*., 1951).

III. RESULTS AND DISCUSSION

Triacylglycerol ester hydrolase activity during longevity insectmoth, *E. vittella* has been studied. Triacylglycerol ester hydrolase activity was maximum at pH 7.8 in male moth and pH 7.7 in female moth. The K_m value was noted to be 0.13 × 10^{-2}mM and 0.20× 10^{-2} mM respectively for male and female moth. The specific activity of triacylglycerol ester hydrolase activity revealed 25.075 and 24.274 µmol FFA /mg protein /25 minutes respectively of 7-day male moth and of 8-day female moth of insect, *E. vittella*.The adult developmental period was found to be 16 and 18 days respectively in male and female of *E. vittella*. The adult developmental period was found to be 16 and 18 days respectively in male and female of *E. vittella*. Lipolytic activity during male and female adult development was shown in figure 1.

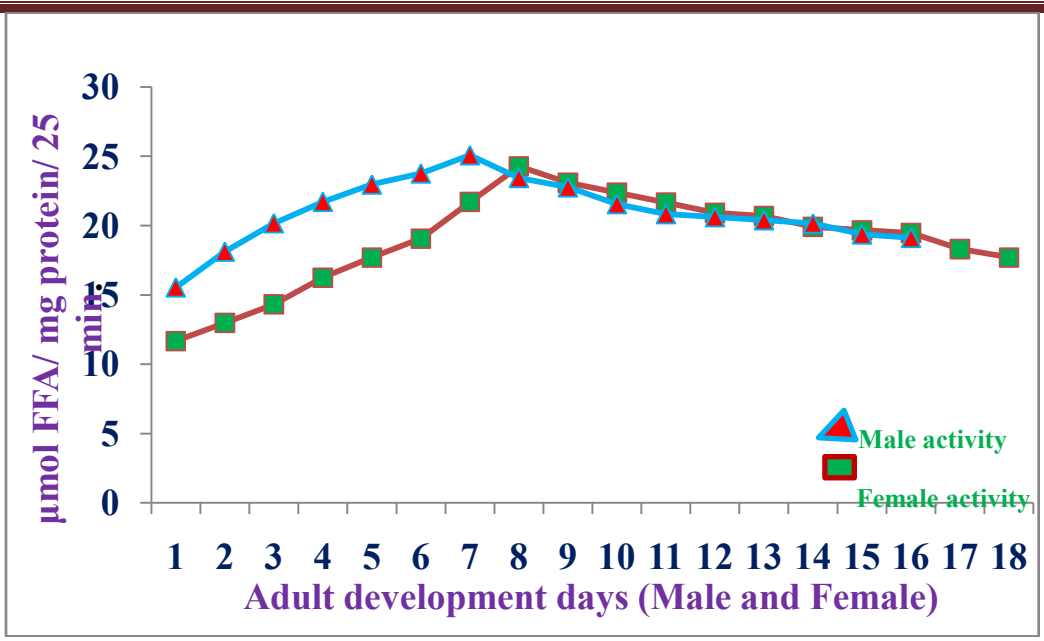

Fig.1:Ttriacylglycerol ester hydrolase activity during longevity of insectmoth, *E. vittella.*

In the adult of *Tramea virgina* optimum lipase enzyme activity recorded at temperature 37 ^{0}C, pH 7 and 0.1% enzyme concentration (Tembhare and Muthal, 1992). Lipase enzyme from *Drosophila melanogaster* indicates highest activity at pH 7 and lipase activity in male was 5 fold higher than female, lipase activity present in male accessory glands of *D. melanogaster* pH 7 (Smith *et al*., 1994). Partially purified phospholipase A_2 from adult midgut of beetle, *Cicindella circumpicta* has pH optima 9 (Uscian *et al.,* 1995). Phospholipase A_2 activity occurs at standard conditions of incubation time 30 minutes, temperature 28 ^{0}C and pH 9 in adult burying beetles, *Nicrophorus marginatus* (Rana *et al*., 1997). Increases in lipase activity of fat body homogenate after initiation of flight have been observed in several insect species (Auerswald and Gade, 2006). Lipid metabolism and role of midgut triacylglycerol lipase in female adult *Rhodnius prolixus* was studied lipase from midgut tissue shows optimum activity at pH 7.0- 7.5 (Grillo, *et al.* 2007). The lipase activity from fat body of adult of 1-2 day emerged insect *M. sexta* was 9 fold higher than larvae (Arrese *et al.,* 2010). Devi and Singh (2011) reported that lipase activity present in foregut, midgut and hindgut of mango weevil, *Sternochetus gravis*. The labial gland extracts of 3rd day male of *Bombus terrestris* shows highest lipolytic activity at pH 8.3 and optimum temperature 50 ^{0}C. The K_m value recorded 0.0011mM and maximum velocity V_{max} value observed 0.15U/mg (Brabcova *et al.,* 2013). The activity of triacylglycerol lipase in Mason bee, *O. rufa* was studied at 37 ^{0}C (Dmochowska *et al.,* 2013).). In bumble bee lipase participate in the hydrolysis of lipids (Brabcova *et al*., 2013).The ratio of the triacylglyceride lipase and diacylglyceride lipase was reoprted in female insects (Weidlich *et al*., 2015).The activity of lipase in silk gland of female *B. mori* was 0.324 µmol (Kumar and Balasubramanian, 2014). In male cockroach, *P. americana* lipase activity was observed in foregut and midgut (Oyebanji *et al.,* 2014). Digestive lipase in dung beetle, *Chironitis arrowi* reveals maximum pH 8, optimum temperature 40 ^{0}C and incubation time 10 minutes (Gaikwad and Bhavane, 2015). Lipase enzyme activity was studied in cricket, *Velarifictorus ornatus* and found higher in long winged males (Lu-quan *et al*., 2015). Lipase activity in the midgut of sunn pest, *Eurygaster integriceps* was studied at temperature 37°C and at pH 8 (Saadati and Mirzaei, 2016). The maximum triacylglycerol lipase activity observed at pH 7.8 which indicates that male moth triacylglycerol lipase is significantly active at alkaline pH. The increased specific triacylglycerol lipase activity25.075 µmol FFA /mg protein /25 minutes from 1- day to 7-day male moth of *E. vittella* suggests more requirement energy for active flights to the male moth in search of female. This increased triacylglycerol lipase activity may be concerned with structural components and sperms production. The decreased in triacylglycerol lipase activity from 7-day male moth to 16-day old male moth of *E. vittella* indicates less active stage of male moth and senescence. The maximum triacylglycerol lipase activity was observed in 7-day old male moth of *E. vittella* indicates most active stage of moth. The main source of energy during male moth ageing is lipid and lipolytic activity. In present study, gradual increase in triacylglycerol lipase activity from 1 to 8 day female adult moths suggest active developmental period requiring maximum energy for oogenesis. The

maximum specific triacylglycerol lipase activity24.274 µmol FFA /mg protein /25 minutes in 8-day female adult moths of *E. vittella* suggested active role of lipase in oogenesis and flight. The decrease in enzyme activity from 8 to 18 day female adult moths indicates inactive stages of adult requiring less energy. Triacylglycerol lipase activity from *E. vittella* revealed rate of release of free fatty acids in the order of Male adult> female adult. In present study, triacylglycerol lipase activity from adult male and female will be helpful for better understanding of its physiology which will lead to new strategies for management of this important pest.

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PROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *ALOE VERA* LEAF GEL ON BLOOD INSULIN LEVEL OF ALLOXAN INDUCEDDIABETIC MICE

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ABSTRACT: *Diabetes mellitus is serious globally increasing health issue. The abnormal metabolism of insulin or absolute deficiency which results in imbalance of glucose metabolism and leads to a syndrome diabetes mellitus. The present work was designed to investigate the protective effect of ethanolic extract of Aloe vera leaf gel on blood insulin level in alloxan induced diabetic mice.*

Mice were devided into four groups. Group I served as control group, group II alloxan induced diabetic group, group III Aloe veratreated group and group IV insulin treated group which is standard antidiabetic drug. The result revealed that there is significant decrease in blood insulin level ($p < 0.01$) in group II but after treatment of ethanolic extract of Aloe vera leaf there is significant increase in blood insulin level ($p < 0.01$) in group III.The result compared with standard antidiabetic drug in group IV.It can optimistically emerge as a new hope in the management of diabetes mellitus.

Keywords: *Diabetes mellitus, Aloe vera, alloxan, insulin.*

I. Introduction

Diabetes mellitus (DM) is a complex group of metabolic disorders which disturbs the metabolism of carbohydrate, protein and fat (Kahn *et al*., 1991; Bliss, 2000). DM classified into two major types Type I which is insulin dependent diabetes mellitus (IDDM) and Type II which is non insulin dependent diabetes mellitus (NIDDM) (Kharroubi and Darwish, 2015). IDDM condition form due to autoimmune destruction of the β cells of pancreas which leads to hyperglycemia (Aikinson MA and maclaren NK, 1994; Takeshi *et al.,*2002; Patel *et al.,* 2012; Tan *et al.,* 2014).Insulin resistance is a pathophysiological condition occurs due to hypertension, central obesity, dyslipidemia

(Karamanou*et al.,* 2016). Alteration in insulin signaling pathway is the main cause of insulin resistance state. Glucose homeostasis is generally associated with the ability of insulin to mediate tissue glucose uptake. Synthetic antidiabetic drugs has adverse side effects (Suzuki et al., 2009; Holt and Kumar, 2010; Wu et al., 2012)

Herbal plants have been used in folk medicine for their protective properties. Recent studies confirmed the insulin sensitivity improvement capacity of many medicinal plants and herbal preparations are useful alternative drugs(Ghorbani, 2013a; Ghorbani, 2013b).

Aloe vera(Family-Xanthorhoeaceae) is also medicinal plant which is native to North Africa. It is stem less plant. Leaves are thick and fleshy,green (Yates, 2002). Margin of leaf is serrated has small white teeth. The flowers are produced in summer on spike up to 90 cm tall.

The aim of present work is to investigate the effect of ethanolic extract of *Aloe vera* gel on blood insulin level in alloxan induced diabetic mice.

II. MATERIALS AND METHODS

Experimental animals

In present experiment healthy adult male mice were used. Adult mice (4 month) weighing about 32 ± 2 were selected. The animals maintained under standard laboratory conditions with 12 hr light and 12 hr dark cycle at temperature of 26^0 C ± 2^0 C in departmental animal house of Rajarambapu Pharmacy College of Kasegao.. The guidelines of CPCSE were followed throughout the experimentation. The animals were housed in aluminium cages having dimensions of 10" x 8" x 5" and allowed to live in groups of 3-4 animals per cage. They were fed with Amrut Mice feed, marketed from Pranav Agro Industries, Pvt. Ltd. Sangli and water *ad libitum.*

Preparation of *Aloe vera*leaf extract (Rajasekaran et al., 2005)

Fresh leaves of *Aloe vera*were collected from college campus of S. G. M. College, Karad. The plant was identified and authenticated by an expert taxonomist Dr. S. R. Yadav, from Botany Department, Shivaji University, Kolhapur, where voucher specimen (KJS -1) of the plant has been deposited.

The fresh leaves of *Aloe vera* were washed thoroughly with water, peel was removed and the pulp was collected. The pulp was lyophilized. Extraction of lyophilized (Cintex CIC 75) material was carried out

by Soxhlet method (Aswar et al., 2011). The extraction was carried out for 24 hrs. The extract obtained was dried at 37^0 C in oven. The yield was stored in refrigerator at 4^0 C until further use. The residual extract was resuspended in distilled water and used in study as per desired concentration when needed.

Experimental design

Adult male mice were divided into four groups containing five animals per group.

GROUP I : CONTROL GROUP (CG)

Adult male mice of this group were injected 0.5 ml of 0.15 M acetate buffer, (pH 5.4) intraperitoneally(ip) once a day for 15 days.

GROUP II :ALLOXAN INDUCED DIABETIC GROUP (AIG)

Induction of Diabetes

Healthy male mice (n=5) were used for induction of diabetes. Mice were starved approximately for 18 hrs before the induction. Diabetes was induced by a single intraperitoneal (i.P.) injection of 120 mg of alloxan monohydrate per kg body weight of animal. A dose of 120 mg/kg body weight dissolved in cold 0.5 ml acetate buffer 0.15 M, (pH 5.4) which was freshly prepared before injection (Fayed et al., 1988 and Helal., 2000 and Syiem et al., 2002). Diabetes was confirmed by using glucometer after 72 hrs of alloxan injection. Mice with glucose levels ≥ 200 mg/dl were isolated and used as diabetic in this study.

GROUP III :*ALOE VERA* TREATED DIABETIC GROUP (ATG)

Treatment of Dibetic group

Treatment was started after 5 days of induction of diabetes and continued for 15 days (Husain *et al*., 2009). During treatment mice from each group were maintained on the same diet and water adlibitum.

GROUP IV :INSULIN TREATED DIABETIC GROUP (ITG)

Insulin treated diabetic group

Insulin was used as standard drug for the treatment diabetic mice was given i.p injection of insulin 0.5 ml daily for 15 days. (Rajesh Mandade,2012)

Estimation of blood insulin

Serum insulin concentrations estimation was carried out at Kale Pathological Laboratary, KaradDist- Satara, MS India by using a fully automated chemi luminescent immune assay kit: Insulin survey (ING): CAP number: 7193856-01 (Flier *et al.,* 1979).

Statistical analysis-

The data was statistically analyzed by One way ANOVA followed by Tukey HSD test. All the values were expressed as mean ± S.E. The difference was considered significant when $p<0.001$.

III. RESULTS AND DISCUSSION

The results on blood insulin level of control mice and the changes in (AIG) and in diabetic mice treated with *Aloe vera*leaf extract and insulin are given in table no. 1 and shown in fig. no. 1

The mean level of blood insulin in the adult male CG was assessed to be 1.34 ± 0.1 µU/ml (1:2, $p<0.01$). In AIG it was observed to be 0.56 ± 0.12 µU/ml. After *Aloe vera* leaf extract (300 mg/kg body weight) treatment in diabetic group it was 1.14 ± 0.09 µU/ml (2:3, $p<0.01$), insulin treated group was 1.16 ± 0.05 µU/ml (2:4, $p<0.01$).

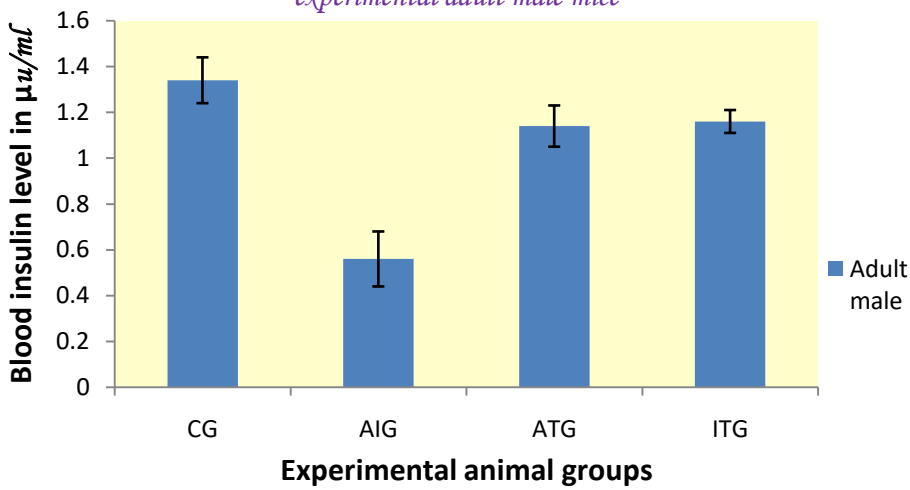
Table 1: showing changes in insulin level in alloxan induced diabetic adult malemice and diabetic mice treated with *Aloe vera* leaf extract and insulin.

Values are mean ± S.D.(Numbers in parenthesis denotes number of animals)

Sr. No.	Group (n=5)	Adult male mice	
		Insulin level (µU/ml)	Statistical significance
1.	CG	1.34 ± 0.1	1:2, P<0.01
2.	AIG	0.56 ± 0.12	2:3, P<0.01
3.	ATG	1.14 ±0.09	2:4, P<0.01
4.	ITG	1.16 ±0.05	3:4, P<0.01

p< 0.05= mostly significant, p < 0.01 =significant, p < 0.001 = highly significant

Fig 1 : showing blood insulin level (µU/ml) in control and experimental adult male mice



Alloxan destroy the pancreatic β cells and inhibit the production of insulin which affect on glucose homeostasis (SaniUM ,2015). In our study it was observed that protective effect of ethanolic extract of *A.vera* leaf gel increased the insulin level significantly. Ethanolic extract of *A. vera* leaf gel extract showed significant regeneration property of β cells. We observed normalization of blood insulin level. The standard drug insulin and ethanolic extract of *A. vera* leaf gel have statistically significant impact. These results are similar to those of previously reported data (Schwartz *et al*.,2016; Paula *et al.,*2017).It is clear that alternative approaches for type I include the stimulation of regeneration of endogenous pancreatic β cells.(Bonner *et al.,*2012).Iranloye*et al*.,2013 studied antidiabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague dawley rats. According to their study Virgin coconut oil improves glucose tolerance by its antioxidant effect which consequently leads to improvement of insulin secretion.Yimam*et al.,* 2014 found the similar result that administration of UP780 improves the activity of β cells might be due to the presence of antioxidant. Takemoto*et al.*, 2014 stated that low insulin concentration induced by alloxan administration ameliorates by feeding the fermented burdock diet.

The results of this experimental study indicate that the ethanolic extract of *A.vera*leaf could have protective effect on blood insulin level. Ethanolic extract of *A. vera* leaf gel contain many chemical compounds, each of which is capable of producing definite biological activities via different mechanism. However further studies are needed to investigate the possible mechanism of action of active compound.

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STUDY OF POPULATION DYNAMICS OF TAPEWORMS IN *GALLUSGALLUSDOMESTICUS* FROM SOLAPUR REGION

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ABSTRACT: *The present investigation deals with the Population Dynamic of tapeworms in Gallusgallusdomesticus from solapur region during October 2013 to September 2014. High infection of Raillietina parasite were occurred in summer season followed by winter season & low in rainy season. This type of results indicates that environment factors & feeding habitats are influencing that parasitic infection either directly or indirectly. This report summarizes the percentages of incidence, intensity, density and index of infection. The present study shows that the seasonal infection of parasites in Gallusgallusdomisticus.*

Keywords: *Gallus gallus domesticus, Population Dynamic, Tapeworm, Solapur.*

I. Introduction

Study of parasites and their relationship to the hosts requires a multidimensional approach in order to understand the nature of parasite and the pathological effects on the hosts. Such studies includes phylogenetic relationship, morphological aspects, ecological aspects, physiology and biochemistry of the parasites and their relationship with their host.

Parasite can have wide range & impact on the ecology of their hosts, in the form of health, (Atme and Owen,1967)behavior (Moore 1984), sexual selection (Howard and Micgella 1990) and regulation of the host population. Chicken is an important source of human food as well as source of economic income. These edible *Gallus gallus domesticus* are infected by number of cestode parasites which cause deteroration in their health, hence their market & nutritive value is decreased.Economic losses are caused by gastrointestinal parasites in a variety of ways.They cause looses through lowered fertility, reduced work capacity, a reduction of food intake & lower weight , increased treatment cost & mortality in heavily parasitized animals.

The present investigation included application of statistical method to understand the distribution of cestode parasites in different season i.e. rainy, winter & summer during the period Oct. 2013 to Sept. 2014.

II. MATERIALS AND METHODS

The intestine of *Gallus gallus domesticus* were collected from chicken market from various places of Solapur district such as Barshi,Vairag,Vadala,Nannaj,Shelgaon during period Oct. 2013 to Sept. 2014 in different seasons.

The intestine of *Gallus gallus domesticus* were dissected longitudinally. Parasite were collected and kept in normal saline (0.9%) solution. Then cestodes were flattened and preserved in hot 4% formalin. These cestodes stained by Harries haematoxyline, washed in distilled water, dehydrated in asending grades of alcohol, cleared in xylene, mounted in D.P.X. Slides were observed under microscope drawings are made with the aid of camera Lucida. The identification was made with the help of "systema Helminthium vol.II," cestode of Vertebrates (Helminths of vertebrates by Yamaguti 1961).

Population dynamics of Cestode Parasites were determined by following formula:

1) Incidence of infection $= \frac{\text{Infected host}}{\text{Total hosts examined}} \times 100$

2) Intensity of infection $= \frac{\text{No .of parasites collected in a sample}}{\text{No .of infected hosts .}}$

3) Density of infection $= \frac{\text{No .of parasite collected in a sample}}{\text{Total host examined .}}$

4) Index G infection $= \frac{\text{No .host infected X No .Parasite collected}}{(\text{Total hosts examined })^2}$

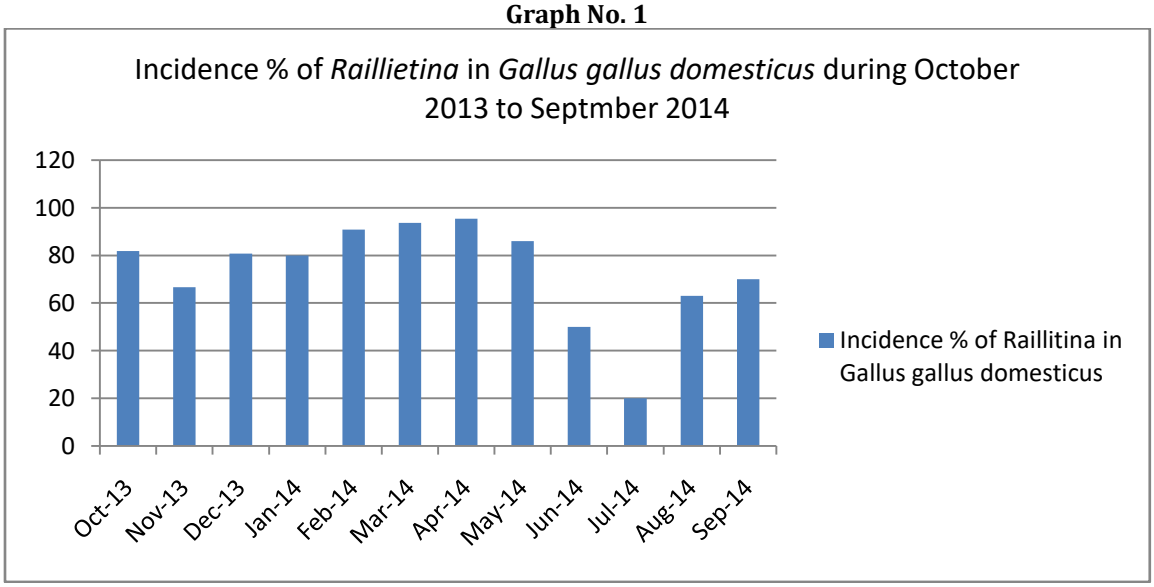
III. RESULTS AND DISCUSSION

The analysis of data shows that the occurrence of *Raillietina*parasites are found in intestine of *Gallus gallus* during variable seasons. (Table no 1 and graph number 1 to 4). The *Raillietina*are found in large number in summer season followed by winter season where as very low in rainy season. The intencity varies greatly due to temperature, humidity and rainfall, feeding habits of host, availability of infective host and parasite maturation. Such factors are responsible for influence of parasite infection. (Kennedy, 1976; Dama *etal* 2012). The values for incidence, intensity, density and index of infection shown in table number 1.

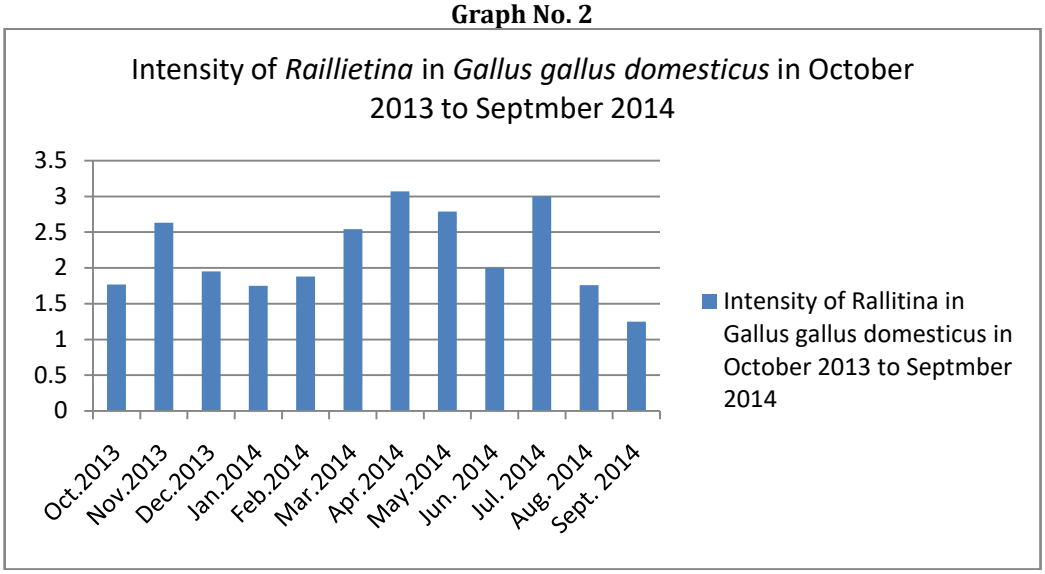
Table No1: Population Dynamics of *Raillietina*in *Gallus gallus* domesticus during Oct.2013 to Sep.2014

Sr. No.	Month	No.of host examines	No.of host infected	No.of Parasite Collected	Incidence %	Intensity	Density	Index of Infection	Locality
1	Oct. 2013	55	45	80	81.81	1.77	1.45	1.19	Pangari
2	Nov. 2013	45	30	79	66.66	2.63	1.75	1.17	Ghari
3	Dec. 2013	52	42	82	80.76	1.95	1.57	1.27	Soudarye
4	Jan. 2014	25	20	35	80	1.75	1.4	1.12	Vadala
5	Feb. 2014	55	50	94	90.90	1.88	1.70	1.61	Tembhurni
6	Mar. 2014	47	44	112	93.61	2.54	2.38	2.23	Barshi
7	Apr. 2014	44	42	129	95.45	3.07	2.93	1.84	Solapur
8	May 2014	50	43	120	86	2.79	2.4	2.06	Vairag
9	Jun. 2014	20	10	20	50	2	1	0.5	Madha
10	Jul. 2014	25	05	15	20	3	0.6	0.12	Khandai
11	Aug. 2014	27	17	30	62.96	1.76	1.11	0.69	Ghari
12	Sept. 2014	40	28	35	70	1.25	0.87	0.61	Pandharpur

Graph No. 1



Graph No. 2



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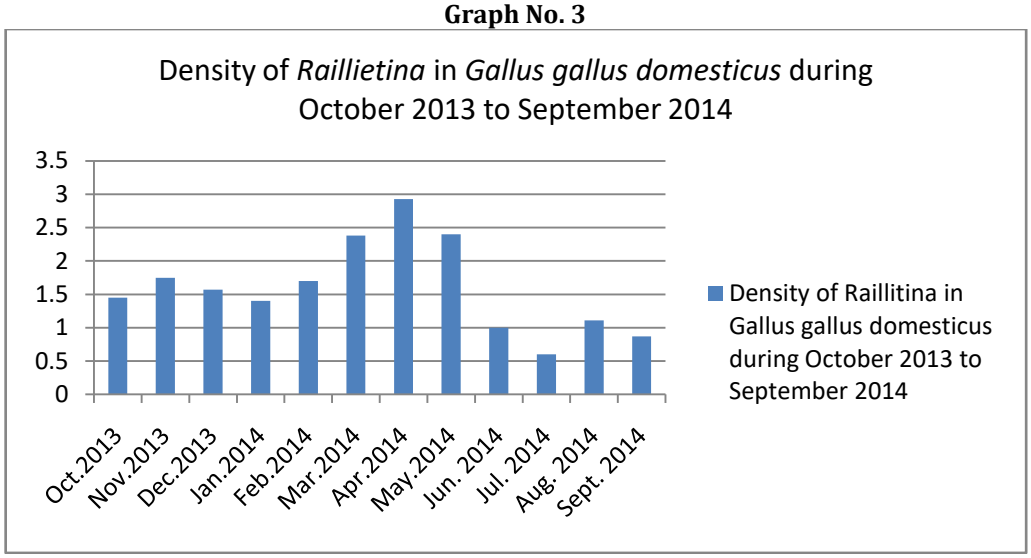
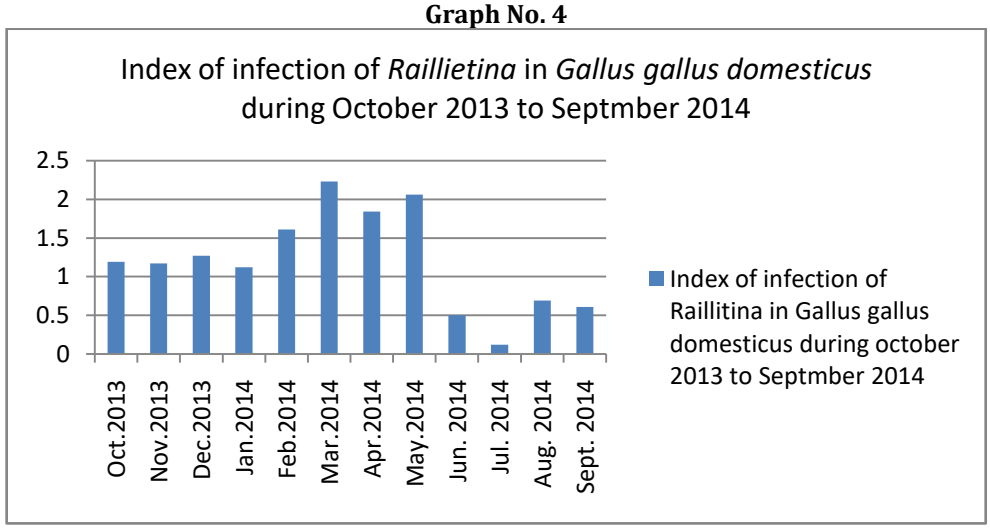


Graph No. 4



IV. CONCLUSION

After the analysis of given data, the present study can be concluded that high prevalence of *Raillietina* (incidences, intensity, density and index of infection), are found in large number during summer season followed by winter season where as very low in rainy season. This type of results indicated that environmental factors and feeding habitat are influencing the parasitic infection either directly or indirectly.

V. ACKNOWLEDGEMENT:

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MONITORING OF THE INSECT POPULATION BY USING MALAISE TRAP MADE UP FROM MOSQUITO NET

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ABSTRACT: *Malaise trap made up from mosquito net is used to monitoring agricultural insect pests belongs orders Hymenoptera, Diptera, Hemiptera, Lepidoptera, Coleoptera, Collembola, Thysanoptera, Neuroptera, Odonata, Ephemeroptera, Orthoptera. This trap is very low cost and effective to monitoragricultural and forest pests as well as for biological pest control agents.*

Keywords: *Mosquito net, malaise trap, agriculture, forest, orders, biological pest control*

I. Introduction

Biodiversity is determined by having a vast knowledge about the taxonomy (Sheikh, 2016). An insect biodiversity account is a largest proportion of all biodiversity on the planet—over half of the estimated 1.5 million organism species described are classified as insects. As insects being the largest group of animals, hence a large number of sampling techniques are employed for their collection. For sampling techniques sweep net, light trap, pitfall trap, Winkler sampling and malaise trap are common and very effective for the collection of different insect groups (Malaise, 1937; Marinoni and Dutra, 1997; New, 1998; Szentkiralyi, 2002; Mason and Bordera, 2008; Aguiar and Santos, 2010; Sheikh et al., 2016). All the insect collection methods above mentioned Malaise trap is commonly used for the sampling of low flying insects (Malaise,1937).

The Malaise trap invented by the Swedish entomologist René Malaise after finding that more insects were captured in his tent than he collected by netting. Malaise traps have wide applications and, when used in a standardized way, can contribute biological and ecological information to a variety of other fields, including taxonomy and systematics, biocontrol and biosecurity such as in forest health monitoring.

Present study the mainly focus on monitoring insect population with malaise trap made up from mosquito net which is very helpful for entomologist, taxonomist and farmers.

II. MATERIALS AND METHODS

Preparation of Malaise trap made up from mosquito net (Fig.1 and 2)

Materials- Mosquito net, empty mineral water bottle, cork of water bottle, alcohol or water and rope.

Method- Many versions of the malaise trap are used, but the basic structure consists of a tent with a large opening at the bottom for insects to fly into and a tall central wall that directs the flying insects upwards to abottle containing aalcohol. Here mosquito nethas been used for trap. Size of mosquito net is length: 2m, width: 1.3m, height: 1.3m, weight: 1.5kg.

One corner of mosquito net has been cut with scissor and attached cork of water bottle with thread. One small hole was made to the empty mineral water bottle so that cork attach to the mosquito net easily fitted to bottle. The bottle must be placed vertically. The bottle has been acts as collector of trap. One face of the insect net has been open and that face towards the crop field so that insects entered in trap.

Trap Location- One of the important part of Malaise trap efficiency is correct placement. The trap should block a corridor or placed perpendicular to a barrier for example crop field border with the collecting head directed to the border and the sun.

Killing Agent- Killing agents though many killing agents have been used for the killing of insects in Malsise traps like Cyanide, 2, 2-dichlorovinyl dimethyl phosphate (Cooksey and Barton, 1981) and others chemicals. However, ethanol is widely used killing agent (Askew, 1980; Brown and Freener, 1995; Cresswell, 1995; Hutcheson, 1999; Gnanakumar et al. 2012; Ghahari and Huang, 2012; Rahman et al., 2016). Priority of a killing agent should depend up on its effectiveness against the target and must have least harm to the nature.For present study 70% alcohol have been used.

Fig. 1- Mosquito net made insect Malaise trap

Fig. 2- Trap with collectormade up from mosquito net applied in field.

Fig. 3 Original malaise trap having cost of Rs. 15000/- (Amazon.com)

III. RESULT AND DISCUSSION

The results were recorded in Table.1 and Figs. 4-6. Trap captured during 15 Sept. to 15 Oct.2018.During study period total no. of insect collected was 806. In this period malaise trap captured 256 Hymenoptera, 156 Diptera, 109 Hemiptera, 50 Lepidoptera, 60 Coleoptera, 20 Collembola, 15 Thysanoptera, 45 Neuroptera, 32 Odonata, 16 Ephemeroptera, 47 Orthoptera. Percentage wise distribution is given in fig. 5. According to percentage Hymenoptera was highest one with 32% and Collembola 2%, Thysanoptera 2%, Ephemeroptera 2% showed lowest percentage. The trap captured depends upon the environmental factors i.e. temperature, humidity, wind and rain fall.

Malaise trap caught insects useful for excellent museum specimens; taxonomists have been usually more willing to make identifications of insect specimen. Temperature, precipitation, air movement and rain fall have been influence the efficiency of Malaise trap operation. The trap captured belongs order Hymenoptera includes Ichneumon wasps, Diptera includes flower flies, Tachinid flies, Coleopteralady beetles, ground beetles, tiger beetles were a very good biocontrol agents. For farmers and researchers mass rearing of above mentioned insect will be very useful.

Advantages of use of trap

1. Mosquito made malaise trap is very low cost equipment. Cost is only Rs. 200 (Amazon.com).
2. This trap reduced man power to control insect pests.
3. Malaise trap is very simple, easily transported, erected and serviced. They will work continuously once set.
4. Do not require a electrical power source.
5. This trap also useful for monitoring changes in seasonal abundance (provided the trap catch is collected weekly).
6. Useful for farmers, researchers, students and entomologist.
7. Easy for washing, low weight trap.
8. We can also used as light trap when provide mercury lamp with power supply.

Disadvantages of use of trap

1. They catch and kill huge numbers of insects.
2. They are affected by strong wind in exposed sites.
3. Needs killing agents.

Table 1. Total Malaise trap captured: comparison of percentage composition, by insect orders

Sr. No.	Order	No. of insect
1.	Hymenoptera	256
2.	Diptera	156
3.	Hemiptera	109
4.	Lepidoptera	50
5.	Coleoptera	60
6.	Collembola	20
7.	Thysanoptera	15
8.	Neuroptera	45
9.	Odonata	32
10.	Ephemeroptera	16
11.	Orthoptera	47

Fig. 6- Chart showing Percentage composition by insect orders.

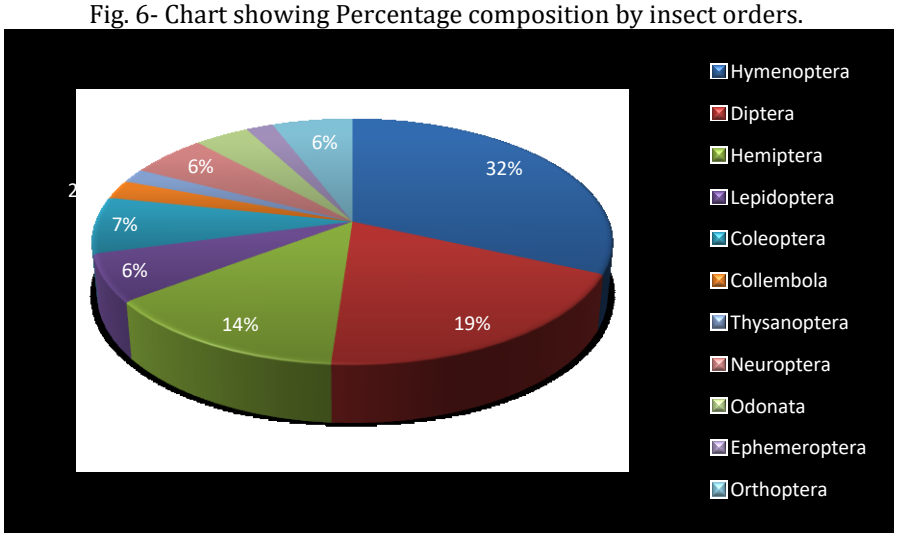
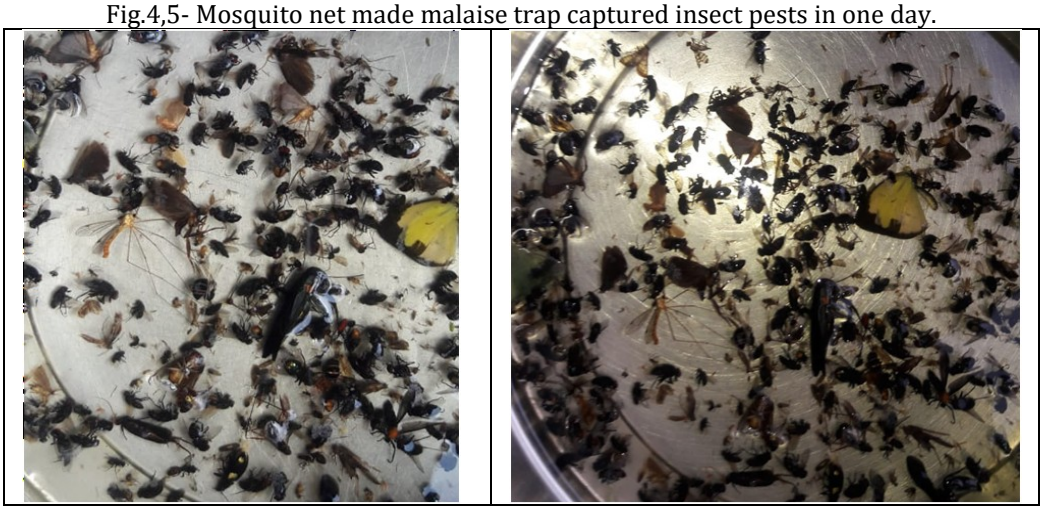


Fig.4,5- Mosquito net made malaise trap captured insect pests in one day.



IV. CONCLUSION

Malaise trap caught insects useful for excellent museum specimens; taxonomists have been usually more willing to make identifications of insect specimen. The trap captured belongs order Hymenoptera includes Ichneumon wasps, Diptera includes flower flies, Tachinid flies, Coleopteralady beetles, ground beetles, tiger beetles were a very good biocontrol agents. For farmers and researchers mass rearing of above mentioned insect will be very useful.

This trap as non-attractant sampling of insect populations offer aefficient and economical means for obtaining large quantities of data with minimaleffort. Hymenoptera Diptera, and Lepidoptera are the most satisfactorily sampled orders,but also catches of various groups. Mosquito net made malaise traps could have additionalvaluable applications in long term faunal composition and seasonality studies, speciesdiversity analyses, and many other ecologically oriented investigations and very low cost.

V. ACKNOWLEDGMENT

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CAFFEINE SUPPLEMENTATION ON CADMIUM INDUCED HISTOPATHOLOGICAL ALTERATIONS IN THE HEPATOPANCREAS OF FRESHWATER BIVALVE, *LAMELLIDENS CORRIANUS (LEA)*

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ABSTRACT: *The present investigation deals with the Population Dynamic of tapeworms in Gallusgallusdomesticus from solapur region during October 2013 to September 2014. High infection of Raillietina parasite were occurred in summer season followed by winter seasThe Study was conducted under five groups of freshwater bivalves Lamellidens corrianus (LEA). Group A was control; B group was exposed to acute dose (LC50/2) cadmium (0.48 ppm) Group C was exposed to acute dose (LC50/2) of cadmium (0.48 ppm) with caffeine (1, 3, 7Trimethylxanthine) (5 mg/l.). After 4 days bivalves from group B were divided into D and E for recovery. D group bivalves pre exposed to acute dose (LC50/2) of cadmium chloride were allowed to cure in normal water. E group bivalves pre exposed to acute dose (LC50/2) of cadmium chloride were exposed to caffeine (5 mg/l.) for recovery. The histological structure of hepatopancreas showed less damage in the presence of caffeine and the recovery from the cadmium induced alterations in the structure of hepatopancreas was faster in presence of caffeine. Which indicates that the caffeine has the capacity to protect the damage of tissues against the arsenic induced toxic impact on & low in rainy season. This type of results indicates that environment factors & feeding habitats are influencing that parasitic infection either directly or indirectly. This report summarizes the percentages of incidence, intensity, density and index of infection. The present study shows that the seasonal infection of parasites in Gallusgallusdomisticus.*

Keywords: *Caffeine; Cadmium; Lamellidens corrianus; Hepatopancreas; Histopathology.*

I. Introduction

The industrial activities of man and the uncontrolled development of large cities, especially during the recent past and combustion of fuels have resulted in the contamination of soil, water and air. The rapid growth of human population and consequent over exploitation of natural resources, industries and tremendous increase in number of vehicles have dumped various hazardous substances in our environment. This has lead to the fast deterioration of environmental quality, ultimately threatening the very existence of healthy life. A large number of chemical substances reach the atmosphere because of natural processes and human activities, most of which may cause serious health problems. U.S. environmental protection agency, occupational safety and health administration and consumer product safety commission have known for centuries as poisons and industrial revolution caused occupational diseases related to exposure variety of toxic metals. The commonest toxic metals found in industrial wastewater are cadmium, chromium, lead, nickel, manganese, mercury, copper and zinc.

Extensive evidence demonstrates that toxicants can disrupt the metabolic, regulatory or disease defense system of an organism, eventually compromising its survival or reproduction for example, genetic damage, malformations, and reduced growth and mobility were observed in pacific herring embryos exposed to PAH levels as low as 0.7 ppb. Arsenic, cadmium, cobalt, chromium, copper, iron, mercury, manganese, molybdenum, nickel, lead, vanadium and show deleterious effects on the water quality, soil quality, enters plants and animals through the food chain, and finally reaches in man in toxic concentration. A new approach to the mechanism of action of heavy metals has been pioneered by Rothstein (1959). It is based on the assumption that the cell membrane is the first point of attack by heavy metal. Most toxic heavy metals are cadmium, mercury, lead that enter the environment and deteriorate water quality. Toxicity of these metals depends on their chemical form, interaction within metals, and the physiochemical parameters like temperature, pH, dissolved oxygen, salinity of water as well as physiological status of the animal and it's acclimation to the metal. In any living tissue, toxicants exert their first effect at the molecular and biochemical levels, and alterations in normal biochemical parameters cause the change in the normal arrangement of molecules and hence the histological aberrations that occur serve as the visual indicators of toxic effects.

Cadmium is a widespread heavy metal in the environment. It is very poisonous, and we only excrete cadmium in very small amounts. Cadmium can cause damage to all types of body cells. By damaging the cell

membrane, cadmium increases the permeability of the cells, one of the consequences because the transfer of other heavy metals into the cells is facilitated. One source of cadmium in our environmentthat bio-accumulate in the body, is tobacco smoke.

Heavy metals enter the system of aquatic organisms via three main pathways. 1) Free metal ions and metal ions adsorbed on the particles that are absorbed through respiratory surface (e.g. gills) readily diffused into the blood stream. 2) Free metal ions that are absorbed by the body surface are passively diffused into the blood stream. 3) Metals that are adsorbed on to food and particulates may be ingested; as well as their free ions are ingested with water (Connell and Miller, 1984).

Caffeine is a drug that is naturally produced in the leaves and seeds of many plants. It is also produced artificially and added to certain foods.Caffeine sources are coffee, tea, cola nuts, etc. The molecule of caffeine being of small molecular weight (194.2), its chelate with heavy metal is easily extractable.Caffeine in combination with an analgesic, such as aspirin, is widely used in the treatment of ordinary types of headache.Canlisk *et.al.*, (2000) studied the effect of drinking beverages with caffeine on bone mineral density (BND) on 77 healthy women, using decaffeinated beverage as control and reported that no change had occurred in BMD of the subjects.

The present investigation is to carried out to study the effect of caffeine on cadmium chloride induced alterations on the histopathology of hepatopancreas of an experimental model, the freshwater bivalve, *Lamellidens corrianus.*

II. MATERIALS AND METHODS

The freshwater bivalve, *Lamellidens corrianus* were collected from the Nathsagar at Paithan. The bivalves were acclimatized to laboratory condition for 3 days, before setting the experiment. Water was changed after every 12 hours and healthy and active animals of approximately same size and weight were chosen.The healthy and active acclimatized bivalves of approximately same size were selected for experiment. These bivalves were divided in to five groups and were treated as follows. Group A bivalves were maintained as Control, group B bivalves were exposed separately to acute doses (LC50values of 96 hr/10) of cadmium chloride (0.48 ppm)up to the 20 days. Group C bivalves were exposed separately to chronic concentration of Cadmium along with of caffeine (5mg/l) up to the20 days. After 20 days exposure to, bivalves from cadmium chloride group B were divided in to two subgroups as D and E for 4 and 8 days recovery studies.Hepatopancreas were fixed in Bouin's fluid for 24 hours, washed and dehydrated in alcohol grades, cleared in toluene and embedded in paraffin wax. Serial sections of 6 µ thickness were cut and stained with Mallory's triple stain. The stained sections were examined under light microscope for histopathological impact of cadmium with and without caffeine and during the recovery.

III. RESULTS

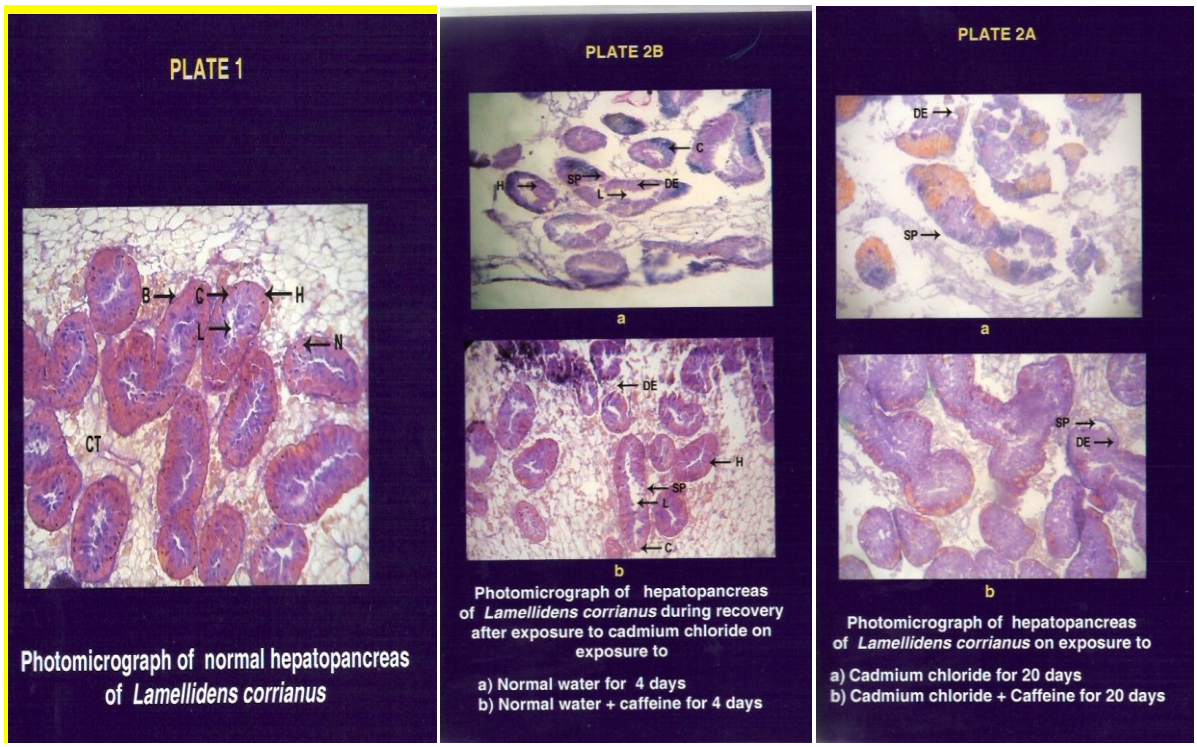
The effect of Cadmium on digestive glands of *Lamellidens corrianus* after exposure to 0.48 ppm of $CdCl_2$ with and without caffeine and during recovery has been shown in the Plate No. 1 to 6 B of the Photomicrographs.

Histological structure of digestive glands (Hepatopancreas)-

Digestive gland is a main organ of storage of metabolic reserves, which provides a source of energy during a period of physiological stress in addition to its secretion for the digestion of food. The hepatopancreas shows hepatic lobules, with columnar cells and secretory cells, both resting on basement membrane. The lobules of glands are bound together by the thin connective tissues layers as shown in photomicroplate 1.

The histological changes in the digestive gland of *Lamellidens corrianus* exposed to cadmium (0.48 ppm) with and without caffeine for 20 days and during recovery are presented in photomicroplate 2A and 2B .After 20 days exposure to Cadmium chloride, the changes in the cytoarchitecture of hepatic lobule of *Lamellidens corrianus* was more severe. During the recovery, the hepatic lobules of hepatopancreas in normal water shows space between epithelium and it's basement membrane, the damaged epithelial cells, increased size of lumen and loss of cytoplasm in cells while the recovery was faster in digestive gland of caffeine exposed bivalves after 4 days. The increase of recovered hepatic lobules of hepatopancreas, of caffeine exposed bivalves indicates that the caffeine increased the rate of recovery as compared to normal water on exposure to cadmium chloride, the hepatic lobules of digestive gland shows swelling. The basement membrane was ruptured, separation of epithelium from the basement membrane, enlargement of columnar epithelial cells and secretary cells, necrosis and vacuolization of cells, increase in the size of

lumen, loss of cytoplasm and degeneration of nucleus of cells were observed after exposure to cadmium. The increase of recovered hepatic lobules in hepatopancreas of caffeine-exposed bivalves indicates that the caffeine increased the rate of recovery as compared to normal water.



IV. DISCUSSION

All the heavy metals possibly affect all the body parts of the treated animals either physiologically, biochemically or by inducing the histopathological changes. There are many evidences, which indicate histopathological changes after heavy metal stress. The abnormalities, which occur at the tissue and cellular level, are the consequences of complex physiological dysfunctions.

In bivalves and gastropods, the digestive gland is the major site of heavy metal storage (Simkiss and Mason, 1983). Owing to the excessive use of pesticides, the environment and water resource are being polluted, thus endangering aquatic life directly and human life indirectly (Gill *et al.,* 1988). Gulbhile (2006) studied mercuric chloride exposure on *Lamellidens Corrianus*, the lamellae of gill showed various changes such as rapture of the ciliated epithelium, increase in the size of lamellae, increase in space between the inter lamellar junction and increase in space between the water tube and inner lamellar junctions. Hosaka et al., (2001) has observed the inhibition of hepatocarcinogenesis by caffeine in Ag1 rats treated with 2-acetylaminoflurene and has proposed that caffeine inhibited hepatocarcinogenesis induced by 2-acetylaminoflure. Mahajan, (2005) investigated that ca Hosaka et al., (2001) has observed the inhibition of hepatocarcinogenesis by caffeine in Ag1 rats treated with 2-acetylaminoflurene and has proposed that caffeine inhibited hepatocarcinogenesis induced by 2-acetylaminoflure. Mahajan, (2005) investigated that caffeine have the capacity to reduce the heavy metals, arsenic trioxide in snail, *Bellamya bengalennsis.* ffeine have the capacity to reduce the heavy metals, arsenic trioxide in snail, *Bellamya bengalennsis.*

Chung Fung – Lung, (1999) suggested that caffeine have the capacity to reduce the tissue damage and protect the hepatopancreas. Puming et al., (2001) studied suppression of lipopolysaccharide induced liver injury by various types of tea and coffee in D-galactosamine sensitized rats and suggested that caffeine containing beverages generally suppress, lipopolyasaccharide induced liver injury according to their caffeine content. The rapidity of this effect suggests that caffeine exerts its effects naturally (Plaskett and Cafarelli, 2001). Inhibition of ATM and ATR kinase activities by the radio sensitizing agent, the caffeine and suggested that the radio sensitizing effects of caffeine are related to inhibition of the protein kinase activities of ATM and ATR and that both proteins are relevant targets for the development of novel anticancer agents (Sarkaria, et al., 1999). Oxygen at second and sixth position of caffeine probably forms the chelate with the

metal and hence caffeine-metal-chelate complex can reduce the activity of metal and complex can be excreted out as it has low molecular weight.

The present investigation indicates that, caffeine has a protective and curative role in the Cadmium inducted alterations. The histological structure of Hepatopancreas showed less damage in the presence of caffeine and the recovery from the cadmium induced alterations in the structure of hepatopancreas was faster in presence of caffeine. The caffeine has the capacity to protect the damage of tissues against the cadmium induced toxic impact.

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IMPAIRMENT OF PULMONARY FUNCTION IN WOMEN EXPOSED TO BIOMASS FUEL SMOKE IN RURAL AREA OF SANGLI DISTRICT (MAHARASHTRA)

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ABSTRACT: *On a global scale the household use of solid fuels is the most important source of indoor pollution and the exposure to the byproducts of combustion of biomass fuel particularly wood smoke has been related to chronic obstructive pulmonary diseases.*

In India 95% households use wood as the primary cooking fuel. Due to easy availability of biomass fuel women from rural area uses biomass fuel such as wood and cow dung for cooking and heating purposes, which in presence of poor ventilation produces a very high level of indoor air pollution. The smoke released due to incomplete combustion of unprocessed solid biomass fuel contains high volume of health damaging air born pollutants. Such as respirable Particulate Matter (PM), Carbon Mono-oxide (CO), Nitrogen Oxide (NO_2), Sulfur dioxide (SO_2) formaldehyde, polycyclic organic hydrocarbons and other some toxic compounds. Inhalation of such air born pollutants causes adverse effect on respiratory system, which reduces lung function and causes Chronic Obstructive Pulmonary Disease (COPD). COPD is the inflammation of bronchi and bronchioles. In COPD if $FEV_1\% < 80$ and $FEV_1\%/FVC\% < 80$ then there is obstructive type of disorder.

In this study, we selected 100 women using biomass fuel and 100 women using LPG from rural area Bisur, which is 6 to 7 km away from Sangli City. Biomass fuel users were considered as Subject and LPG users were considered as control. Survey of women using chulla and LPG was done. Information regarding type of house, type of kitchen, number of years and number of hours exposed to biomass fuel and LPG was collected. Spirometry was done in total 200 women. Spirometric parameters Forced Expiratory Volume per one second ($FEV_1\%$), Forced Vital Capacity (FVC%) and ratio of Forced Expiratory Volume per one second / Forced Vital Capacity (FEV_1/ FVC%) were recorded. We found, out of 100 subject women using chulla 40 women had $FEV_1\% < 80\%$ and ratio of $FEV_1\%/FVC\% < 80\%$. In 40 women had pulmonary impairment that is obstructive type of disorder.

Keywords: *Biomass Fuel, $FEV_1\%$ (Forced Expiratory Volume per one second), Forced Expiratory Volume per one second / forced vital capacity, COPD.*

I. Introduction

More than half of the world's populations rely on unprocessed solid fuels for cooking and heating (Bruce *et al.;* 2000 and Kiraz *et al.;* 2003). In the developing countries in the South Asia and Sub-Saharan Africa 80% population rely on unprocessed solid fuel (Holdren and Smith.; 2000). Poor house holds in Asian and Latin America depend on fuel wood. Nearly two billion Kg of biomass are burned every day in economically poor courtiers. (Barnes *et al.;* 1994).

Majority of rural homes in India are pucca or semipucca type. Most of the homes have closed kitchen without proper ventilation. Where as some of the homes, have no closed kitchens where cooking is done in open area. The most important role in the life of average Indian housewife is the domestic cooking. The life of typical Indian house holds revolve around the cooking area where Indian women spent much of the time there. For daily cooking Indian house wife spent on an average more than 6 hours daily in the kitchen for cooking food. Indian woman is exposed to the biomass fuel smoke at early age of 15 yrs. During her lifetime she is exposed to biomass fuel for 30 to 40 yrs, equivalent to 60,000 hrs. The type of house, location of kitchen and type of fuel used play a significant role on women health.

Cooking is the most important activity contributing to indoor air pollution.Indoor air pollution from biomass burning in India alone register over 6,00,000 premature deaths per year that can be attributed to biomass fuel use (Smith *et al*.; 2002).

The use of biomass fuels mainly wood has been associated with an impairment of pulmonary function. Mild to moderate reductions of FEV_1/FVC, FEV%. Combustion of biomass produces a large amount of smoke that spreads into the environment as air pollutants. Exposure to such biomass smoke causes adverse effect on respiratory system. Biomass fuel smoke is the most important risk factor for COPD where

indoor ventilation is inefficient (Albalak *et al.;* 1997, De Koning *et al.*; 1985).

In India 95% households use wood as the primary cooking fuel. Due to easy availability of biomass fuel women from rural area uses biomass fuel such as wood and cow dung for cooking and heating purposes,which in presence of poor ventilation produces a very high level of indoor air pollution. The smoke released due to incomplete combustion of unprocessed solid biomass fuel contains high volume of health damaging air born pollutants. Such as respirable Particulate Matter (PM), Carbon Mono-oxide (CO), Nitrogen Oxide (NO_2), Sulfur dioxide (SO_2) formaldehyde, polycyclic organic hydrocarbons and other some toxic compounds. Inhalation of such air born pollutants causes adverse effect on respiratory system, which reduces lung function and causes Chronic Obstructive Pulmonary Disease (COPD). COPD is the inflammation of bronchi and bronchioles. In COPD if $FEV_1\% < 80$ and $FEV_1\%/FVC\% < 80$ then there is obstructive type of disorder.

Objectives:

I. Survey of women using chulla and LPG from rural area Bisur of Sangli district.

II. To estimate forced expiratory volume per one second in percentage, ($FEV_1\%$), Forced Vital Capacity in percentage (FVC%) and Ratio of forced expiratory volume per one second and Forced Vital Capacity in Percentage ($FEV_1/FVC\%$).

II. MATERIALS AND METHODS

For this study we selected Bisur rural area of Sangli district, which is 6 to 8 km away from Sangli City. Survey of women using chulla and LPG was done from this rural area. We selected 100 women using chulla and LPG. Biomass fuel users were considered as Subject and LPG users were considered as control. All women participated in this study are above 35 yrs of age and are from low socio economic status. Information regarding age, height, weight and type of house, type of kitchen, number of hours and number of years exposed to biomass fuel and LPG was collected. Spirometery was done in total 200 women. Spirometric parameters Forced Expiratory Volume in One Second ($FEV_1\%$), Forced Vital Capacity (FVC%) and ratio of $FEV_1/FEV\%$ were recorded.

Statistical analysis (Gupta and Kappor, 1983)Calculated Z test based on null hypothesis was done. Cal |Z| = > table Z = 1.96 at 5% level of significance. If Z value is greater than table value 1.96 then there is significance difference between control and subject.

III. OBSERVATIONS

Table No. 1
Survey of LPG using women (n=100) as per House Type, Kitchen Type, Exposure Time, Exposure Year, Literacy.

Survey	House type	Kitchen type	Hours Exposure		Years of Exposure		Literacy	
	Concrete	Indoor	>4	<4	>15	<15	Literate	Illiterate
LPG	100	100	76	24	73	27	97	3
Total	100	100	100		100		100	

From Table No.1 it is observed that 100 women using LPG, live in concrete house and use indoor kitchen. From this 76 women using LPG for greater than 4 years and 24 women using LPG less than 4 years. 73 women using LPG for more than 15 years and 27 women using LPG less than 15 years. From control group 97 women are literate and 3 women is illiterate.

Table No. 2
Survey of Biomass fuel using women (n=100) as per Use of fuel, Type of House, Exposure time, Exposure years, Literacy.

Survey	Women using Type of Fuel		Women using House type		Women using indoor Kitchen	No. of women Exposed for years		No. of women Exposed for hours per day		Data of women Literacy	
	Wood	Wood + Dung	Kutcha	Semikutcha		> 15 yrs	< 15 yrs	>6 hrs	<6 hrs	Literate	Illiterate
Biomass fuel	83	17	97	3	100	89	11	85	15	15	85
Total	100		100		100	100		100		100	

From Table No. 2 in subject group it is observed that 83 women using Biomass fuel, wood and 17 women using wood and dung. 97 women living in kutcha type of houses and 3 women in semikutcha type of house. Total 100 subject women using indoor kitchen. 89 women exposed to biomass fuel for more than 15 years and 11 women exposed to biomass fuel for less than 15 years. 85 women exposed to biomass fuel for greater than 6 hours and 15 women exposed to biomass fuel for less than 6 hours. In subject group 85 women were illiterate and 15 women were literate.

Table No. – 3
Data of Spirometry (FEV_1%, FVC%, FEV_1/FVC%) of Rural Women from Bisur Exposed to Biomass smoke

Sr. No.	CONTROL					SUBJECT				
	Age	Years	FEV_1%	FVC%	FEV_1/ FVC%	Age	Years	FEV_1%	FVC%	FEV_1/ FVC%
Mean	41.29	20.83	102.71	95.91	86.73	42.50	21.69	73.29	73.16	81.91
Var.	14.47	34.50	224.76	233.02	55.58	24.51	26.11	713.88	511.11	87.08
Sqrt	0.62	0.78	3.06	2.73	1.19					
Z	-1.94	-1.10	9.60	8.34	4.04					

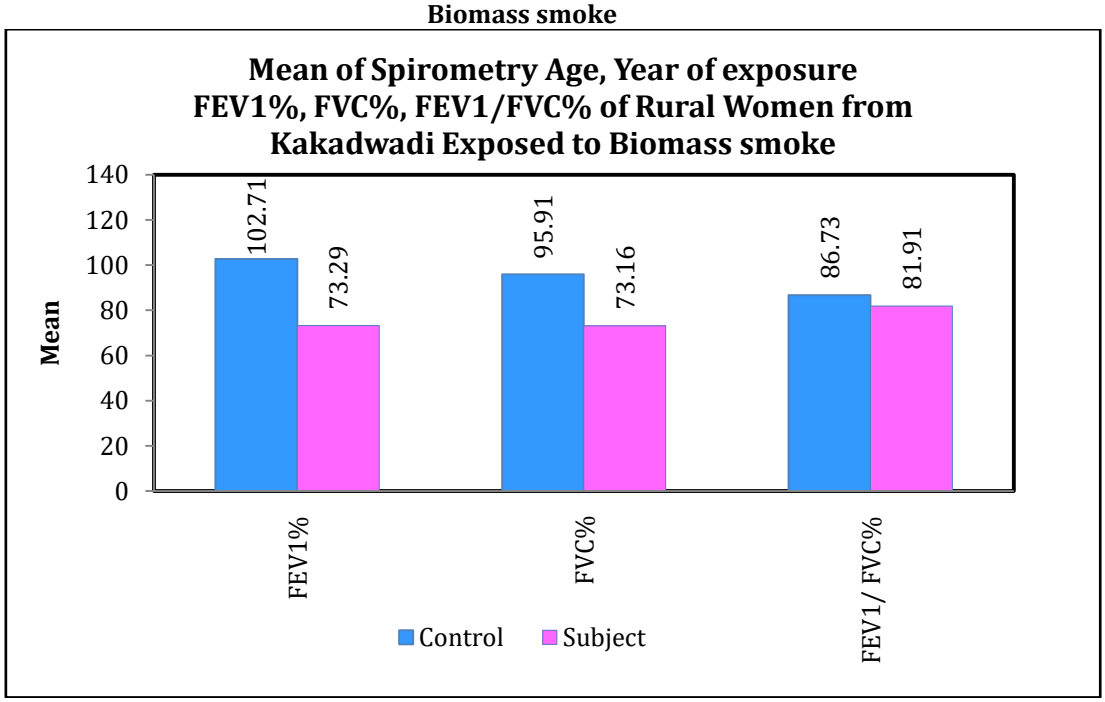
Observations on spirometry (in percentage) of women exposed to biomass fuel from Bisur village

Table No.3 represents observations on Age, years of exposure and spirometry (in percentage) of control and Subject women in the village Bisur. 100 women using LPG (Control) and 100 women using chulla (Subject) from village Bisur were selected for the study of spirometry. The values of Age, years of exposure and the values of FEV_1%, FVC%, FEV_1/FVC% in percentage of each control and Subject women were recorded in Table No. 3. The mean values and Z values of Age, years of exposure and FEV_1%, FVC%, FEV_1/FVC% from this table are as below.

The mean values of Age and years of exposure of control women are 41.29 and20.83. While mean values of Age and year of exposure of subject women are 42.50 and 21.69. The mean values of FEV_1%, FVC%, FEV_1/FVC%, of control women are 102.71, 95.91 and 86.73 respectively, the mean values of FEV_1%, FVC%, FEV_1/FVC% of Subject women are 73.29, 73.16 and 81.91 respectively. These values are shown at the base of each column in the Table No. 3.

The calculated Z value of Age and years of exposure and calculated Z value of FEV_1%, FVC%, FEV_1/FVC% based on null hypothesis are at the last of each column in the Table No.3. The calculated Z value of Age and years of exposure are 1.94 and 1.10. The calculated Z values of Age and years of exposure are less than table value 1.96 hence there is no significant difference in age and year of exposure of control and subject women. While calculated Z values of FEV_1%, FVC% and FEV_1/FVC% are 9.60, 8.34 and 4.04 respectively. The calculated Z values of FEV_1%, FVC%, FEV_1/FVC% are greater than table value 1.96 hence there is significant difference inFEV_1%, FVC%, FEV_1/FVC% of control and Subject women. The result is significant at 5% level of significance.

Figure No. 1:Data of Spirometry (FEV_1%, FVC%, FEV_1/FVC%) of Rural Women from Bisur Exposed to Biomass smoke



IV. RESULT AND DISCUSSION

In present study out of 100 subject women 40 women had FEV_1% < 80% and ration of FEV_1% / FVC% < 80%. In the rural area of Bisur, women from low socio economic status uses biomass fuel wood and dung. Majority of women exposed to biomass fuel smoke more than 15 yrs and more than 6 hrs per day. These women suffered from COPD which is obstructive type of disorder.

In obstructive type of spirometry pattern there is narrowing of small airway due to chronic inflammation. According to Dennis *et al.* (1996), Orozco *et al.* (2006), Caballero *et al.* (2006) the reduction in FEV_1% and FEV_1%/ FVC% may be due to chronic inhalation of toxic substance emitted during biomass combustion leading to inflammatory changes in (bronchi and bronchioles) FEV_1% < 80% and ration of Dutt *et al.* (1996) reported that the parameters FEV_1% and FEV_1%/ FVC% was significantly lower in biofuel users compared with both kerosene and LPG users.

Mangat *et al.* (2013) studied pulmonary function tests in rural women exposed to biomass fuel and reported that the lung function parameters FEV_1% and FEV_1/FVC% were significantly lower in the study group exposed to biomass fuel than control.

Similar types of results are observed in present investigation. In this study, 40 women out of 100 women had FEV_1% < 80% and FEV_1/FVC% < 80%. The results of spirometry of subject and control group shows that there was significant difference in the 'Z' values of parameters of spirometry (FEV_1% and FEV_1/FVC%). Statistical analysis showed that 'Z' values of FEV_1% and FEV_1/FVC% were significantly reduced in subject group as compared to control group.

V. CONCLUSION

- Prolonged exposure to biomass fuel smoke in poorly ventilated kitchen causes reduced lung functions and women suffered from Chronic Obstructive Pulmonary Diseases (COPD).
- In subject group the type of COPD observed was obstructive.
- As age and years of exposure increases COPD increases.

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EFFECT OF NOVEL INSECTICIDE CHLORANTRANILIPROLE ON LIPID PEROXIDATION OF FINGERLINGS OF FRESHWATER FISH *CIRRHINUS MRIGALA*

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ABSTRACT: *Chlorantraniliprole is a novel anthranilicdiamide insecticide and is widely used to control agricultural pests in the Order Lepidopteron, Coleopteran, Dipterans and Hemiptera species. Most of the insecticides arrive at the surrounding water bodies and affects the normal flora and fauna of the aquatic ecosystem is due to runoff rain water and soil erosion which gets accumulated. Toxic chemicals induce oxidative damage, which increases free radicals in the body of exposed organisms. The increased free radicals oxidatively damage the lipids of the cell membrane of many tissues and are known as Lipid Peroxidation. The present study was designed to evaluate the toxicity of Chlorantraniliprole on Lipid Peroxidation in fingerlings of freshwater fish Cirrhinus mrigala. Prior to experimental protocol, fingerlings were acclimatized in glass aquarium for 07 days. After acclimatization, fingerlings were exposed to predetermined LC_0 and LC_{50} concentration of Chlorantraniliprole in twenty liter test container for 96 hrs (static bio assay method). In the present study, it was observed that the Lipid Peroxidation in gills, muscle,liverand brain were significantly increased in LC_0 and LC_{50} concentration as compared to the control group, which in turn concludes that the selected insecticide Chlorantraniliprole, do interferes and causes deterioration changes in selected test fish Cirrhinus mrigala.*

Keywords: *Lipid Peroxidation, Chlorantraniliprole, Cirrhinus mrigala.*

I. Introduction

Chlorantraniliprole is a new commercial synthetic insecticide(3-Bromo- 4'-chloro-1-(3-chloro-2-pyridyl)-2'-methyl-6'-(methylcarbamoyl)pyrazole-5 carboxanilide).It is useful against large varieties of the lepidopteronand few species of Coleoptera, Diptera and Hemiptera. It is belongs to the ryanodine receptor modulator, which is an intracellular calcium channel in various tissues(Truong and Pessah, 2018). Chlorantraniliprole triggers the release and reduction of intracellular calcium stores from the sarcoplasmic reticulum of muscle cells by activating the insect ryanodine receptors (Truong and Pessah, 2018). It causes impaired muscle regulation, paralysis and ultimately death of sensitive species.Pesticides are operating consistently in the agricultural field to control insects of crops and animals. These are used in various phases in agriculture field. Most of the insecticides arrive at the surrounding water bodies such as rivers, lake and agricultural ponds due to runoff rain water and soil erosion and gets accumulated. These affect the normal flora and fauna of the aquatic ecosystem.

It is well known fact that, large amount of pesticides are accumulated in the aquatic bodies. Fishes are highly sensitive to the alterations in the quality of water and very vulnerable to such environmental stresses. Numerous studies demonstrated that the pesticide in water bodies induces oxidative stress (Abdollahi*et al*., 2004). The oxidative stress is imbalance between the production of reactive oxygen species (ROS) and antioxidant defense system in the tissue of exposed animals (Mittler, 2002). That results into production of numerous free radicals through several biochemical mechanisms. Such common free radicals are*hydroxyl radical*, *superoxide anion* radical, hydrogen *peroxide*, oxygen singlet, hypochlorite, *nitric oxide, etc. (*Cadenas E, Davies., 2000*).*The increased concentration of free radicals induces the damaging effect to exposed organism and impairs the antioxidant system (Parvez and Raisuddin, 2005).

The hydroxyl radical is one of themost free radical which induces lipid peroxidation (Gutteridge*et al*., 1979). This radical is produced in degradation of $H_2O_2$by antioxidant enzymes such as superoxide dismutase and catalase (Hogg, 1992). These produced hydroxyl radicals attack on lipid molecules, but mostly on polyunsaturated fatty acids (Bielski*et al*., 1983). That can be led to a chain of reaction of the lipid peroxidation and produced many aldehyde compounds such as malondialdehyde (MDA) (Janero, 1990). It is the end product of lipid peroxidation. These produced malondialdehydeoxidatively reacts with other biomolecules such as proteins which leads to alteration in structure and function (Berlett*et al*., 1997).

Therefore, the present study is designed to evaluate the toxicity of Chlorantraniliprole on Lipid Peroxidation in fingerlings of freshwater fish *Cirrhinus mrigala*.

II. MATERIALS AND METHODS

Collection and Acclimatization of the Specimen

The freshwater fish *Cirrhinus mrigala* were collected from government fish seed production and rearing centre Dhom, Dist. Satara. Prior to the acclimatization in laboratory condition, fishes were offering a bath with disinfectant 0.05% $KMnO_4$ solution toavert bruise and disease. After disinfection fish were maintained in glass aquariawith supplying continuous aeration. During the span of acclimatization, the water was changed daily to discard remaining food particles and faeces, and the specimens were fed every day with fish food(TAIYO Pet Products Pvt Ltd, India).

Toxicity Test

The insecticide chlorantraniliprole (98%) buy from M/S Super Bio Tech Marketing Company,India. The clear and de-chlorinated water was used for acclimatization and experimentation. A well-acclimatized healthy fish measuring 6±8 cm in length and 9±11g in weight were selected for the present study. The toxicity was carried out in 20 liter plastic trough. In each trough 10 fish were released. The trough was distinguished in three groups 1) Control group (Without any exposure of Chlorantraniliprole)2) LC_0 Concentration group(Exposed to 0.0025ppm concentration of Chlorantraniliprole) 3) LC_{50} concentration group(Exposed to 0.01ppm concentration of Chlorantraniliprole). After 24hrs, experimental medium was replaced by fresh medium.

Lipid Peroxidation Analysis

After96 hoursexposures, the fishesfrom the control group, LC_0 and LC_{50} concentration groupwere sacrificed. The gill, muscle, liver and brainwere separated from sacrificedfishes and lipid peroxidation was estimated by will (1966) method. Simply, Homogenate of each tissue was prepared in 1ml reaction mixture (Phosphate buffer solution pH 7.4 containing 75mM ascorbic acid and 1mM $FeCl_3$). For the lipid peroxidation triplet set of testtubes were prepared for control group, LC_0 Concentration group and LC_{50} concentration.Then the 0.2 ml homogenate was added in each test tube.After that, 1.8 ml distilled water; 1 ml 20% TCA(Trichloroacetic acid), 2ml 0.67% TBA (Thiobarbic acid) was added. The blank was prepared by adding 2 ml distilled water, 1 ml 20% TCA, 2ml 0.67% TBA. Then all tubes kept in boiling water bath for 15 minutes. Thereafter, tubes were cooled and centrifuged at 3,000 rpm for 10 minute. The absorbance value was measured at 532nm by spectrophotometrically. The amount of malondialdehyde was calculated by following formula:

Malondialdehyde /mg tissue = O.D of a sample/ 0.156 X 2nM of MDA/mg tissue.

Where,

0.156 = the absorbance for 1mM solution of malondialdehyde in a 1cm thick cell at 532nm

2 = amount of tissue taken in mg present in 0.2 ml of a sample.

Statistical analysis

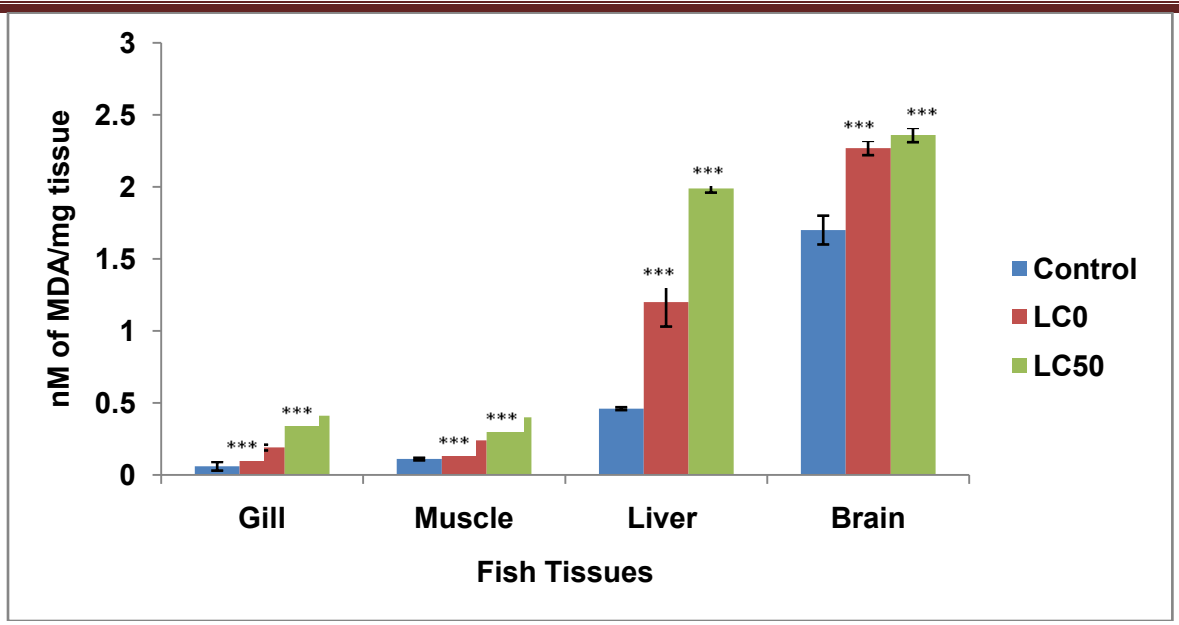
The observed data from each group were expressed in arithmetic mean ± standard deviation. The level of significance was calculated using unpaired student's t test.

III. RESULTS AND DISCUSSION

Table: Effect of Chlorantraniliprole on the lipid peroxidation in various organs of the fish *Cirrhinus mrigala* after acute exposure

Groups	Amount of MDA in nM/mg tissue			
	Gill	Muscle	Liver	Brain
Control Group	0.06 ± 0.03	0.11 ± 0.009	0.46±0.01	1.70±0.10
LC_0	0.19 ± 0.02	0.24 ± 0.03	1.20±0.17	2.27±0.05
LC_{50}	0.41 ± 0.02	0.40 ± 0.02	1.99±0.03	2.36±0.05

Results expressed as arithmetic mean ± standard deviation, *** indicates $p< 0.0001$.



Graph: Activity of lipid peroxidation on gill, muscle, liver and brain of the fish *Cirrhinus mrigala*after acute exposure (96 hours) of Chlorantraniliprole.

Data expressed in arithmetic mean ± standard deviation. Bars represent SD of six observed individual observations. *** indicates $p< 0.0001$

The result of the effect of Chlorantraniliprole on the lipid peroxidation in various organs viz. gill, muscle, liver and brain of the fish *Cirrhinus mrigala* in the control group,LC_0 concentration group and LC_{50} concentration group after acute exposure (96 hours) are depicted in following Table and represented graphically. In the gill of control group, the level of lipid peroxidation was 0.06 ± 0.03nM of MDA/mg tissue. However, in LC_0 concentration group fish gill exhibited 0.19 ± 0.02 nM of MDA/mg tissue and in the LC_{50} concentration group fish showed 0.41 ± 0.02nM of MDA/mg tissue of gill. In the control group fishes, 0.11 ± 0.009 nM of MDA/mg tissue of the muscle, while in the LC_0 concentration group fishes exhibited 0.24 ± 0.03nM of MDA/mg tissue of muscle and in the LC_{50} concentration group fishes it was 0.40 ± 0.02nM of MDA/mg tissue of muscle. The amount of MDA in the liver tissue of the control group was 0.46 ± 0.01 nM of MDA/mg tissue. However, 1.20 ± 0.17nM of MDA/mg tissue of liver and 1.99 ± 0.03 nM of MDA/mg tissue of liver were in the LC_0 concentration group fishes and LC_{50} concentration fish group respectively. The brain of control group fish exhibited 1.70 ± 0.10nM of MDA/mg tissue. While, in the LC_0 concentration group fish showed 2.27 ± 0.05 nMof MDA/mg tissue of brain and in the LC_{50} concentration group was 2.36 ± 0.05 nM of MDA/mg tissue of brain. The amount of MDA in the gill, muscle, liver and brain tissues after exposure of 96 hours of Chlorantraniliprole at LC_0 and LC_{50} concentration increased as compared to the control group. The difference was highly significant at $p<0.001$. The amount of MDA in the brain of LC_0 and LC_{50} concentration fishes were more as compared to the other tissues.

Numerous pesticides induced the biochemical changes in the carbohydrates, proteins and lipids. This results in the altered metabolic activity and normal functioning of exposed organisms. The intensity of toxicant above the threshold level induces the mortality of the exposed organisms. Lipid peroxidation is nothing but the oxidative damage to lipids. Malondialdehyde is the end product of lipid peroxidation, which measured in nM of MDA. The amount of MDA is directly proportional to the lipid peroxidation in the tissues. Wong-Ekkabut et al., (2007) demonstrated that the increased lipid peroxidation altered the physiological function of the cell and tissues and induces cellular membrane damages. In the present study it is observed that, the acute exposure (96 hours) of Chlorantraniliprole at LC_0 and LC_{50} concentration induces lipid peroxidation. The lipid peroxidation was significantly increased in the LC_0 concentration group fishes and LC_{50} concentration exposed group fish as compared to control group fishes which were never exposed to any toxicants. The lipid peroxidation in the brain and liver is elevated as compared to gill and muscle tissues, moreover much higher in the brain tissue.

Bantu *et al.,*(2013) demonstrated that, exposure of*Labeorohita* for 15 days and 30 day to the sub lethal concentration of Chlorantraniliprole increased the lipid peroxidation in liver, kidney, gills, and muscle tissues. They also observed that increased the antioxidant activity such as SOD and CAT activity in the liver,

kidney, gills, and muscle. Thus, the similar results were observed in the present study after exposure of 96 hours in the freshwater fish *Cirrhinus mrigala*.Increased antioxidant level in the tissues indicates the increased oxidative damage. Ercal et al., (2001) and Büyükokuroğlu et al., (2002) observed that increased the level of lipid peroxidation in the tissues is the indicator of oxidative damage. In the present investigation, the level of lipid peroxidation in the LC_0 and LC_{50}exposed fish were increased. This indicates that the Chlorantraniliprole might be inducing the oxidative damage.

Friedman (2010) revealed that the nervous system was more vulnerable to oxidative stress because high oxygen consumption that leads to higher production of free radicals. Furthermore, in the brain tissue rich in polyunsaturated fatty acids which are more vulnerable to free radical attack (Friedman 2010). In the present study, the increased lipid peroxidation in the brain of LC_0 and LC_{50} concentration of Chlorantraniliproleexposed fishes were might be due to more amounts of polyunsaturated fatty acids in the brain.

IV. CONCLUSION

It can be concluded that, the increased MDA level in the gill, muscle, liver and brain tissue of the fish *Cirrhinus mrigala* exposed to pre determined values of toxicant, indicates that the fishes were under oxidative stress. Chlorantraniliprole is responsible for production of free radicals in the metabolism of fishes *Cirrhinus mrigala.*

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ON A RECORD OF EXOTIC FISH SPECIES *ATRACTOSTEUS SPATULA* (LEPISOSTEIFORMES: LEPISOSTEIDAE) FROM THE FRESHWATER WELL OF KOLHAPUR, MAHARASHTRA, INDIA.

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ABSTRACT: *This paper reported the occurrence of the exotic North American alligator garAtractosteus spatula (Lepisosteidae) in the well of Uchgaon region in Kolhapurcity, Maharashtra. The caught specimen is 657 mm in total length, denotes the first record belongs to the family Lepisosteidae. Probably, the existence of this species might be the result of simply release by local people through aquarium trade way.*

Keywords: *Exotic, Atractosteus Spatula, Occurrence, Aquarium trade*

SHORT NOTE

There are numerous threats reported with regards to the freshwater biodiversity and can be categorized into five interacting groups namely overexploitation, water pollution, flow modification, habitat destruction and the introduction of exotic species (Dudgeon et al., 2006). Among all these threats,invasion of exotic species is the second most cause of local species destruction (Simberloff, 2003). This might bethe exotic species become powerful predators or opponents of indigenous species (Hill and Lodge, 1999), transfers parasites (Torchin and Mitchell, 2004), cause cross hybridization (Mallet, 2007) and might be result ofdecline in native biodiversity by eradicating local species (Dick and Platvoet, 2000).

Presently Indian aquarium has 300 species of exotic fishesthose imported and sold without any regulation (Knight, 2010). In spite of the guidelines of import issued by Government of India and Ministry of Agriculture it is totally failed to stop the introduction of exotics into the natural waters or ecosystems of India (Bijukumar et al., 2013). Possibly, in the present study reported species *Atractosteus spatula* might be the result of this uncontrolled aquarium trade. This is first scientific study reported the existence of exotic species in the natural water. Scare information available in relation to the existence or occurrence or successful invasion population. In India, the occurrence of *Atractosteus spatula* reported from various locations like Telangana, Andhra Pradesh and more recently from Pavana Dam Lake near Pune, Maharashtraand all these reports are from newspapers or blocks on internet.

A single individual of alligator gar native to North America, *Atractosteus spatula* (Lacepede, 1803) was captured fromthe well of Uchgaon region of Kolhapur city, Maharashtra. The specimen was captured with the help of local fisherman using gill net (45mm) in January, 2017. The species identity was confirmed by following morphological keys of Page and Burr (2011) (Table. 1 and 2). The total length is 657 mm while total weight is about 4980gm (Fig. No. 1).

Fortunately this individual found in the freshwater well, however if this recorded into the open water system like nearer Panchganga river, possibly major threat to the native fish biota might be observed due to its feeding habitat (Mutlak et al., 2017). Knowing this threat is the first step towards the conservation strategies. Currently most of the exotic species found in the wild is recorded as aquarium or ornamental species (Luque et al., 2013). Hence, there is necessity to aware the local aquarium traders as well as the buyer about the ecological impact of the present and other exotic species should be undertaken.

Table 1. – Morphometric characteristics of *Atractosteus spatula*

Characteristics	Values
Weight	4980 gm
Total length (TL)	657 mm
Standard length (SL)	539mm
Body depth	126mm
Head length (HL)	239mm
Head depth	73.3mm

Head width	81.9mm
Snout length	125mm
Eye diameter	19.8mm
Interorbital distance	66.5mm
Dorsal fin length	45.7mm
Pectoral fin length	75.1mm
Pelvic fin length	77.8mm
Anal fin length	46.2mm
Predorsal fin length	667mm
Postdorsal fin length	730mm

Table 2. – Merstic characteristics of *Atractosteus spatula*

Characters	**Count**
Dorsal fin rays	7
Anal fin rays	7
Pectoral fin rays	14
Pelvic fin rays	7
Lateral line scales	60

Fig No 1. *Atractosteus spatula*, 657 mm TL collected from well of Uchgaon, Kolhapur

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STUDY OF PHYSICAL FITNESS INDEX AND BODY MASS INDEX IN FEMALE STUDENTS IN S. G. M. COLLEGE, KARAD (M.S.) INDIA

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ABSTRACT: *Physical fitness is ability to carry out daily task with vigour and alertness without getting undue fatigue. Physical fitness is generally achieved through correct nutrition, exercise, hygiene and rest. In present study total 60 female students from S.G.M. College, Karad campus were selected to carryout physical fitness index by Harward step test method. BMI was also recorded by measuring height and weight of student. Study shows that 73% students were having low average PFI value. Individuals with good PFI value are only 1%.This study shows that this may be due to the sedentary life style and lack of sporting activities. Regular physical activity is an important determinant of physical fitness.*

Keywords: *PFI value, BMI,Physical activity.*

I. Introduction

Physical fitness is a state of health and well-being. It is the ability to perform a given set of physical activities or daily task with vigour and alertness without getting undue fatigue. Best fitness indicates the capacity of individual to perform a sedentary task efficiently but also a sense of physical well-being.Physiological fitness implies the capacityfor skilful performance and rapid recovery (Shashiala andGeetanjali, 2014)

There are many psychological, behavioural and physiological reasons as to why people do not perform physical activity or perform limitedly (Bulut, 2013).It is an important problem that physical activity is not performed regularly and insufficiently in many countries. Although people have more spare time now, they still do not allocate sufficient time for physical activity (Heyward, 2006).There has been a decrease in physical activity due to a more sedentary lifestyle. (Karandikar MS, Prasad NB, Asit Kumar).Today, physical activity deficiency is commonly seen in adult and old population.

Physical fitness is the result of regular exercise, proper diet and nutrition, and proper rest for physical recovery. (Parmar, 2015)Physical fitness in healthy personswould depend upon several factors such as the body mass and other factors.

Thelevels of physical fitness are generally assessed by anthropometric measurements such as the body weight, height, chest size and other for recruiting candidates to services such as security, police, army and other services.Harvard step test (HST) which is a common method used to assess cardio - respiratory fitness. (Karandikar,2014)

Poor physical fitness will result in the increasing incidences of various health problems such as cancer, obesity, cardiovascular diseases, and diabetes mellitus. Some of these diseases are leading cause of deaths in the world (Powell K.E. and Blair S.N., 1994).

Present study was carried out at S.G.M. College, Karad campus. Female candidates were subjected to carry out Harward Step Test.BMI of each candidate was calculated. Correlation between PFI and BMI were studied.

II. MATERIALS AND METHODS

A total of 60 were female candidates whose age varied from 18 to 25 years were selected for this study from S.G.M. College, Karad.

A. **Study design:** observational study.
B. **Sample size**: 60 subjects.
C. **Sampling**: convenient sampling method,
D. **Study setting:** SGM campus, Karad.
E. **Inclusive criteria:** Female students age between 18 to 25 years.
F. **Exclusive Criteria:** Individuals with health problems such as respiratory problems, cardiovascular problems, endocrine problems and obesity, history of Major surgery in the recent past.

All the subjects were familiarized with Harvard step test. The procedure of this method is that subject took rest for 5 minutes before test and initial pulse rate was noted. The subject was asked to perform the exercise

of ascending and descending Harvard step (Brouha et al, 1943).
If one gets exhausted earlier then the time was noted for which she was able to perform the test. Time was noted with the help of stopwatch. At the end of test the subject was asked to sit immediately on chair and the pulse rate was counted and recorded during 1 to 1-1/2 min to 2 to 2/-1/2, 3 to 3/-1/2 min interval. Total of these three readings were taken which is called recovery pulse. The duration of exercise was converted in seconds and Harvard index (PFI) was calculated as follows.

$$PFI = \frac{\text{Duration of exercise in seconds}}{2 \times \text{recovery pulse}} \times 100$$

Prior to the test age, height and weight were recorded.

Table: 1. Physical Fitness Index rating.

Male	>90	80-89	65-79	55-64	<55
Female	>86	76-86	61-75	50-60	<50
Grade	Excellent	Good	High average	Low average	Poor

Body mass index was calculated after measuring each subject's body weight in kilograms and height in meter.

BMI was calculated using formula

$$BMI = \frac{\text{Weight (Kg)}}{(\text{Height in meter})^2}$$

Table:2 Categories of subjects depending upon their BMI value. (Nutritional status & BMI, WHO)

BMI value	<18.5	18.5-24.9	25-29.9	>30
Category	Under weight	Normal weight	Over weight /preobesity	Obese

III. RESULTS AND DISCUSSION

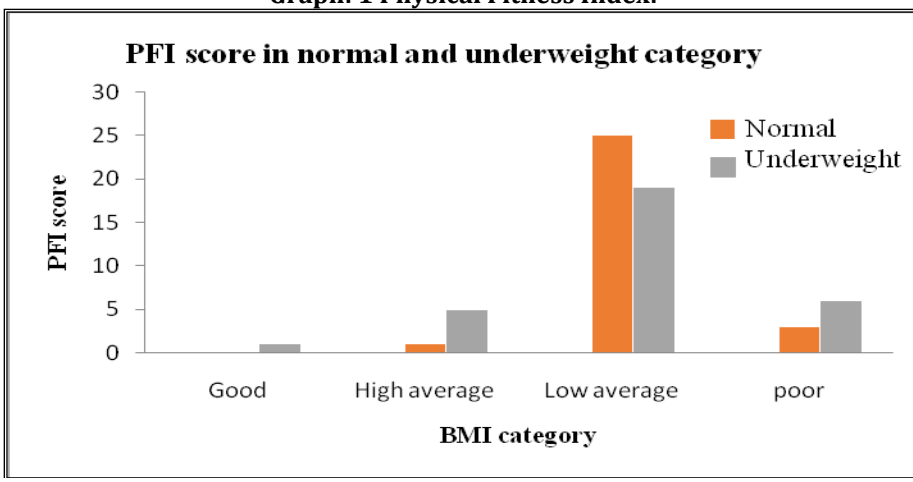
Table:3 Profile of subjects

Group	Age	N	Height	Weight	BMI*	PFI*	r value
Total	20.22±1.63	60	1.57 ±0.05	45.32 ±6.15	18.34 ±2.07	55.48 ±6.52	-0.22056
Normal	20.71±1.76	29	1.58 ±0.06	49.59 ±4.90	20.05 ±1.35	54.86 ±5.78	-0.37768
Underweight	19.73±1.31	31	1.55 ±0.05	41.31 ±4.20	16.76 ±1.10	56.09 ±7.20	-0.13254

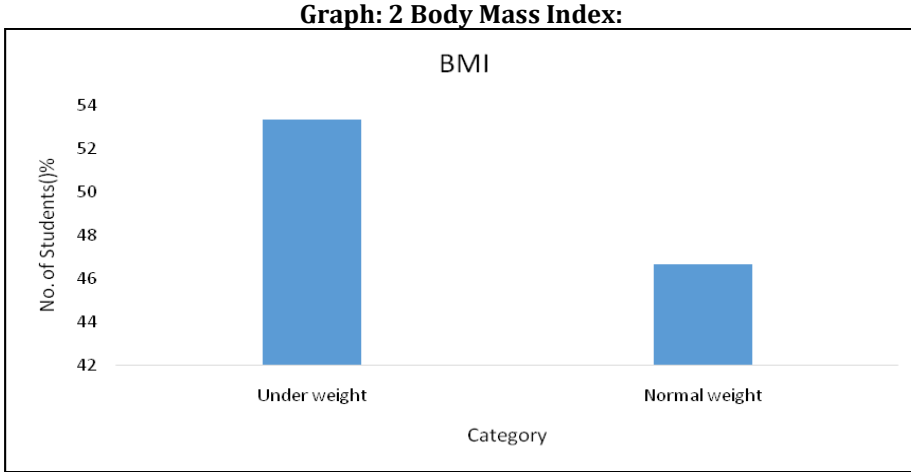
*: Mean ± SD

Figure 1.Female candidate performing Harvard step test

Graph: 1 Physical Fitness Index:



Graph: 2 Body Mass Index:



The results of the present study reveal that the cardio vascular fitness of young individuals from 18 to 25 years of age is not satisfactory. Only 73% girls are having low average grade of PFI, whereas 10% with high average and 1% with good PFI value. BMI values indicate that 53.33% girls are under weight while 46.66% girls are normal weight category. They have low cardiovascular endurance and hence fitness.

Several studies have established that physical fitness is necessary to carry out daily task. The effect of regular exercise is known to have beneficial effect on health. Akre,Ambarish & Bhimani, Neha(2015) studied the co- relation between physical fitness index (PFI) and body mass index in asymptomatic college girls and showed that there was a negative correlation between the physical fitness index and body mass index.Shrivastav et al(2013)conducted a similar study on 22 young subjects in the age group of 18 -25 years which concluded that there was a negative significant correlation between BMI and aerobic fitness (r= -0.55).

These results correlate with other studies that researched the same variables (Graf et al., 2004,Chen, et al. 2006,Tokmakidis, et al. 2006). The overweight and obesity are associated with lowered muscle strength *(Tokmakidis et al, 2006).* There was a negative significant correlation between health-related anthropometric measures and physical fitness factors as per Leila Jaafari et al (2012).

Physical Fitness Index depends on recovery of the heart rate after exercise and therefore is seen to be less in the overweight group as the overweight group has a higher resting heart rate due to altered sympathetic activity and also the return to resting is prevented by the altered function of the sympathetic nervous system. (Akre, Ambarish & Bhimani, Neha2015)

IV. CONCLUSION

There is a negative correlation between Physical Fitness Index and Body Mass Index. From BMI

value it is clear that approximately half of the girl students are underweight that may lead to various health problems. Regular physical activity is an important determinant of physical fitness.Overweightness decreases physical fitness.

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STUDIES ON PRIMARY PRODUCTIVITY OF RIVER GODAVARI AT GOWARDHAN GHAT, NANDED

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ABSTRACT: *The total amount of plant organic material fixed in the form of producers is called primary productivity. The rate at which the photosynthetic and chemosynthetic activities capture the solar energy and store them in the form of organic substance is called as primary productivity of the water body. Productivity is a living factor; producing capacity of a water body, the living components of any ecosystem consists of producer and consumer.*

Average gross primary production ranged from 0.49mgc /m3/hr in January and March and 0.67mgc/m3/hr in February. The net primary production ranged from 0.25mgc/m3/hr in January to 0.29mgc/m3/hr in March.

Keywords: *Primary productivity, chemosynthetic, river Godavari.*

I. Introduction

The study of primary productivity helps in finding self sustaining capacity of the water body. Odum (1956, 1937) calculated the primary productivity of thermal spring by using diurnal changes in dissolved oxygen content. Singh and Singh (1990) evaluated the productivity of Ganga. Sreenivasan (1972) and Kannan and Job (1980) studied productivity of reservoirs in Tamil Nadu. Birsal (1996) recorded the primary productivity of Supa Reservoir. While Manjaramkar *et. al*. (2012) evaluated primary productivity of Government fish seed farm, Siddheshwar Camp.

The total amount of plant organic material fixed in the form of producers is called primary production. The rate at which the photosynthetic and chemosynthetic activities captured the solar energy and store them in the form of organic substance is called as primary productivity of water body.

After careful observation of the available literature, it was decided to study the primary productivity of river Godawari at Gowardhan Ghat. River Godawari is holy river called as, "Deccan Ganges". It is one of the most important river of East coast river system of India, originates from Deolali Hills at Trimbakeshwar, near Nashik, Maharashtra State. River flows through the states like Maharashtra, Telangana and Andhra Pradesh and empties its water in Bay of Bengal below Rajahmundry in Andhra Pradesh. In Maharashtra is flows through the districts like Nashik, Ahmad nagar, Beed, Aurangabad, Parbhani and Nanded.

Gowardhan Ghat is located in Nanded city, which is most polluted area, where sewage water and funeral wastes are dumped in river Godawari. The fish fauna of river Godawari at Gowardhan Ghat to Nagina Ghat mostly composed of carnivorous fishes. Herbivorous fishes also found rarely in this area hence it is decided to study the primary productivity of this area.

II. MATERIALS AND METHODS

Present investigation was undertaken on river Godawari at Gowardhan Ghat area. The exact location of study station was below the Gowardhan Ghat Bridge. The primary productivity was estimated by using light and dark bottle method as described by Trivedy and Goel (1986).

Monthly samples were collected from study station from the month of July 2017 to June 2018. The bottles were suspended in the river with the stakes and where kept for incubation for 6 hours, from 10.00 AM to 04.00 PM. The dissolved oxygen was estimated immediately from initial bottle. After incubation dissolved oxygen was estimated in light and dark bottle by Winkler's method as described by Saharan *et. al.* (2002). Primary productivity (Gross and Net production) was estimated by following formula:

$$\text{Gross primary production (mgc/ m}^3\text{/ hr)} = \frac{(C3-C2) \times 0.375}{1.25 \times 6}$$

$$\text{Net primary production (mgc/ m}^3\text{/ hr)} = \frac{(C3-C1) \times 0.375}{1.25 \times 6}$$

Where, C1= DO content of initial bottle
C2= DO content of light bottle
C3= DO content of Dark bottle

The results were expressed in mgc/ m^3/ hr.

III. RESULTS AND DISCUSSION

Results of present investigation are depicted in table below:

Sr. No.	Month	Gross Production (mgc/ m^3/ hr)	Net Production (mgc/ m^3/ hr)
1	July 2017	0.34	0.16
2	August 2017	0.18	0.32
3	September 2017	0.84	0.14
4	October 2017	0.84	0.36
5	November 2017	0.27	0.16
6	December 2017	0.24	0.11
7	January 2018	0.76	0.42
8	February 2018	0.81	0.37
9	March 2018	0.86	0.22
10	April 2018	0.30	0.21
11	May 2018	0.33	0.38
12	June 2018	0.84	0.28

Gross primary production ranged from 0.18 mgc/ m^3/ hr to 0.86 mgc/ m^3/ hr, minimum during the month of August and maximum during March. The Net primary production ranged from 0.11 mgc/ m^3/ hr to 0.42 mgc/ m^3/ hr, minimum during the month of December and maximum during the month of January.

Goldman and Witzel (1963) reported values of productivity where bimodal in Clear Lake of California. Kaff (1963) noted similar pattern in Arctic pond.

Bhagat (1973) recorded maximum value in monsoon and post monsoon and minimum in summer. Kulkarni (2002), noted maximum values of productivity during November and minimum values during September at Derala tank. Manjaramkar *et. al*. (2012) noted maximum value of productivity during monsoon, while average minimum value in summer months from Government fish seed farm Sidheshwar.

From present investigation it is evident that the maximum values of primary productivity during post monsoon months and minimum values of productivity during summer months. It can be concluded from present study that the study station is not suitable for dispersal of herbivorous fishes.

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HOST SPECIFICITY IN *ACTIA NIGROSCUTELLATA*

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ABSTRACT: *Tachinids exploit a wide diversity of hosts belonging to many orders and families of insects. S. litutra was attacked by parasitoid A. nigroscutellata as this pest species is polyphagous and found in various agro and forest ecosysytems. While, H. armigera although it also found in various agro and forest ecosysytems, attacked by less number of parasitoids (only one in present study) indicating that the hosts has developed strong defense against present tachnids. Likewise A. janata also not attacked by A. nigroscutellata. The most suitable host (with 90% parasitism) for A. nigroscutellata was S. litutra. The host species H. armigera and A. janata remain unparasitized. While, S. exigua showed 40% parasitism. Maximum 48 parasitoids have been emerged from 10 hosts of S. litutra. Suggesting it suitability in mass rearing of this parasitoid.*

Keywords: *A. nigroscutellata, host specificity, parasitism, mass rearing.*

I. Introduction

Host specificity studies are great concern to many researchers in entomophagous insects. Since, they are used as biocontrol agents in pest management (Sathe, 2015). Accordingly to Salt (1935) host selection phenomenon by parasitoid consists of four distinct steps namely habitat selection, host location, host acceptance and host suitability. Host suitability is further dependant upon morphological and physiological adaptations both in hosts and parasitoids. The apparent lability of host use among most Tachinidae may be due to a general lack of host specific adaptations relating to host physiological defenses (Stireman et. al 2005).

Tachinids exploit a wide diversity of hosts belonging to many orders and families of insects. The strictest association between tachinid and host groups are the restriction of phasiinae to Heteroptera and Rutiliini to scarab hosts. However, some broad parasitoid host association refer to Dexiinae-Scarabaeidae, Tachninae-Lepidoptera, Exoristinae-Lepidoptera and ormiini-Orthoptera. The wide spread use of Lepidoptera in Exoristinae, Tachninae and Dexiinae suggests that members of this order may have served as ancestral hosts of tachnids. Most tachnids attack exophytic caterpillars and other holometabolous larvae which have similaritis with caterpillars such as sawflies and chrysomelids. Tachnids have breathing tubes to maintain direct contact with atmospheric air via spiracles.

Host associations of tachnids are not strongly limited by physiological suitability or host defenses (Stireman et. al 2005). The processes of host location and selection that determine proximate host use may ultimately shape broad scale ecological and evolutionary patterns of host use. The mechanism by which most tachnids locate and select hosts are not well understood. However certain parasitoids are more amenable to laboratory production than others, their mass production is simplified on factitious hosts and can be commercialized on large scale for pest management. Keeping in all above facts, present topic was selected.

In past Gutierrez (1970), Lingren et. al (1970), Lingren & Noble (1972), Lewis & vinson (1971, 1975), Drooz and Fedde (1972), Calvert (1973) Jackson et. al (1979), Hopper & King (1984), Sathe & Jadhav (2001), etc attempted studies pertaining to host-parasitoid specificity.

II. MATERIALS AND METHODS

Laboratory cultures of hosts and parasitoids were used in the present experiments. Each experiment was replicated for five times. *S. litutra*, *S. exigua*, *H. armigera*, and *A. janata* larvae of host density 10 of known age 8 days were exposed to parasitoids *A. nudibasis* and *A. nigroscutellata* for 24 hours in glass cages for parasitization. After oviposition by parasitoids, the parasitized larvae were reared separately in plastic containers for parasitoid/moth emergence. Later, percent parasitism was calculated. During the experiments parasitoids fed with 50% honey and host larvae with mulberry leaves except *A. janata* on caster leaves.

III. RESULTS

Table 1: Host specificity in *A. nigroscutellata*

Sr. No.	Host species	Host density exposed	Parasotoids emerged	Moths emerged	% parasitism
1	*S. litutra*	10	48	1	90.00
2	*S. exigua*	10	20	6	40.00
3	*H. armigera*	10	0.0	10	0.00
4	*A. janata*	10	0.0	10	0.00

Host specificity in *A. nigroscutellata*:

Results recorded in table. 1 & fig 1-6 indicates that the most suitable host (with 90% parasitism) for *A. nigroscutellata* was *S. litutra*. The host species *H. armigera* and *A. janata* remain unparasitized. While, *S. exigua* showed 40% parasitism. Maximum 48 parasitoids have been emerged from 10 hosts of *S. litutra*. Suggesting it suitability in mass rearing of this parasitoid.

Fig. 2- host specificity in *A. nigroscutellata.*

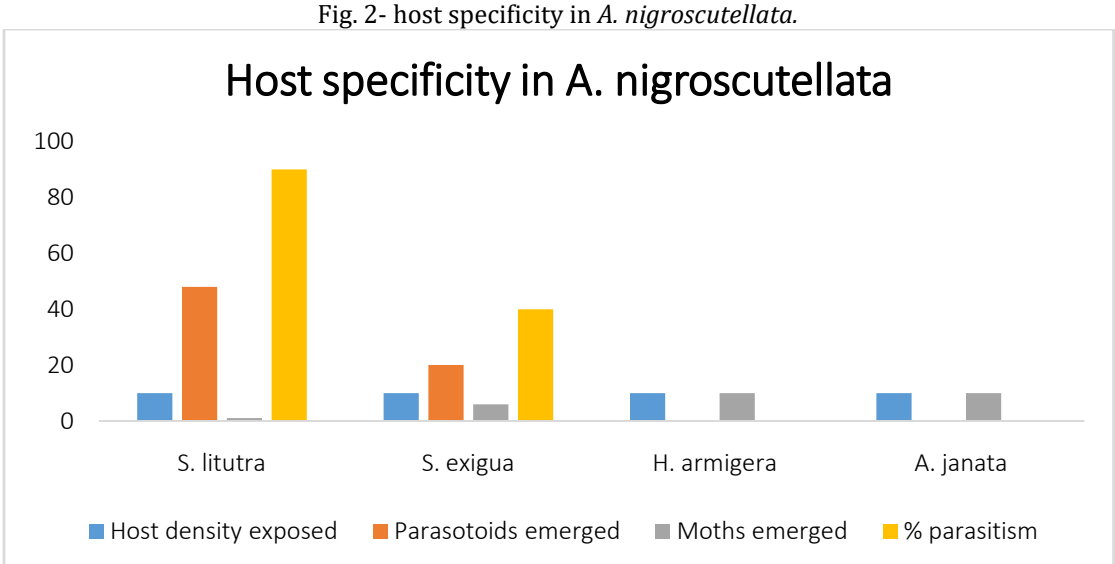

Fig. 3- *A. nigroscutellata*emergence from different host larvae (n=30).

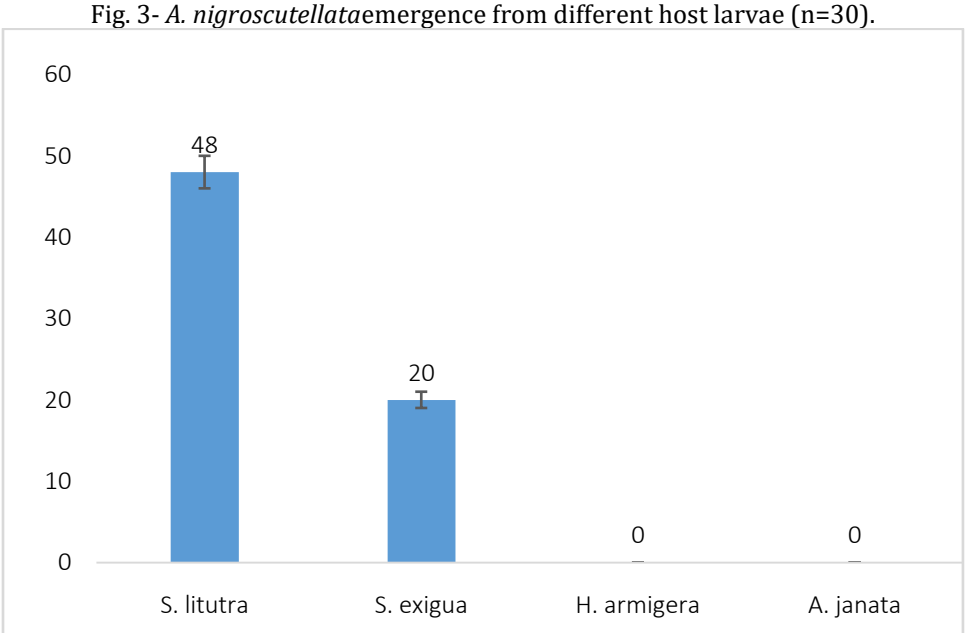

Fig. 4 Percent parasitism of *A. nigroscutellata* from different host larvae.

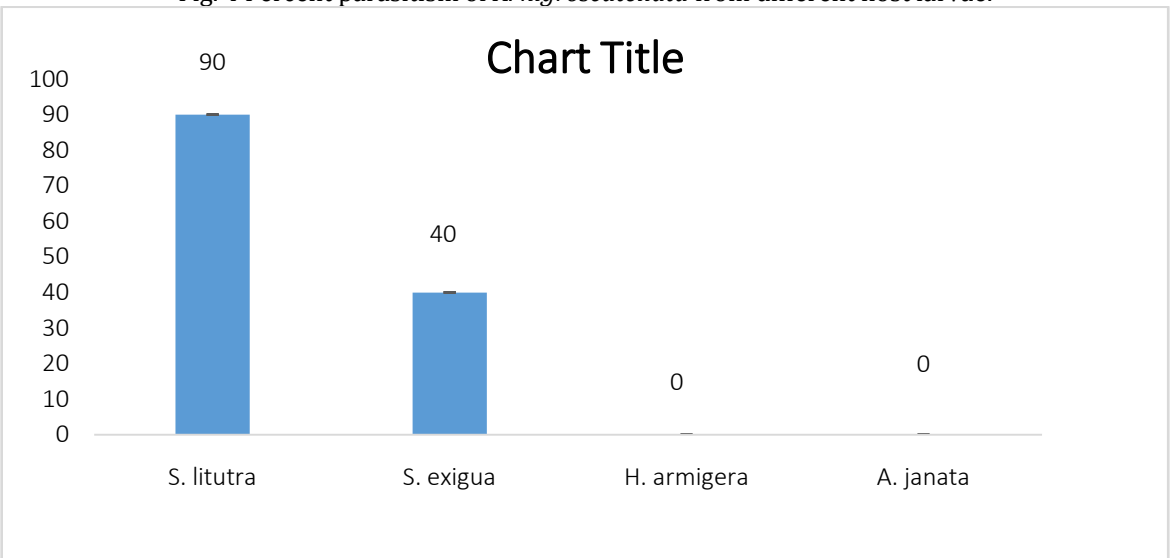

Fig. 5 Larvae of *H. armigera*

Fig.6 *A. nigroscutellata*(parasitoid)

IV. DISCUSSION

According to Eggleton & Gaston (1992) the set of hosts tachnids attack may be more closely related to their ecology than to their phylogenetic affinities. While, Stireman (2005) says that host range is not strongly conserved and not closely related to oviposition strategies or other major traits that define clades within tachnidae. In the present work *S. litutra* was attacked by parasitoid *A. nigroscutellata* as this pest species is polyphagous and found in various agro and forest ecosysytems. While, *H. armigera* although it also found in various agro and forest ecosysytems, attacked by less number of parasitoids (only one in present study) indicating that the hosts has developed strong defense against present tachnids. Likewise *A. janata* also not attacked by *A. nigroscutellata*. Studies in the present work indicating it strong defenses may be morphological or physiological needs to investigate this aspect as further avenue for research in entomophagae.

Eleven host larval species have been tried against *E. Bombycis* for their parasitization by Narayan swamy *et. al* (1993). Their results indicated that the parasitoid preferred *B. mori* with 54.52%, *amia cynthia recini* boisdual 13.22%, *Antheraea mylitta* D. 10.00%, *S. litutra* 8.9%, *H. armigera* 7.41%, *A. janata* 5.16%, and *Adisura atkinsoni* Moore 0.86%. While, *Spilosoma obliqua* (Walker) *Euproctis fraterna* M., *Eupterote mollifera* W. and *Attera fabricialla* remain unattacked. They concluded that texture of the skin, physical obstructions and also body size of hosts influenced the rate of oviposition by parasitoids.

According to Beeson & Chatterjee (1935) *T. bombycis* can attack several lepidopterous hosts which includes *Acherontia Lachesis* F., *Attacus ricini* Boisdual, *Dasychira mendosa* Hubner, *D. thwaitesti* Moore, *Thiocides postica* Walker, and *Metanastrio hyrtaca* Cameron. Crosskey (1977) given host parasitoid index of Tachinids of oriental region. *Tachina sorbillans* was studied with respect to host specificity by Isarangkul *et. al* (1972) from Bangladesh. *A. janata* and *Prodenia litura* Fab were important hosts they reported. While, Sabrosky & Reardon (1976) reported *Lymantria dispar* L. as new host to *T. sorbillans*. Similarly Siddappaji &

Jamil (1992) and Thompson (1950) separately recorded 44 host species for uzifly. Supplementary hosts are essential in mass rearing of parasitoids. Hence, the present work has special importance in biological pest control programme.

V. ACKNOWLEDGMENT:

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PHOTOPERIOD INDUCED ALTERATIONS IN GROWTH PATTERN OF SILKWORM*BOMBYX MORI* (L.) REARED ON M_5 AND V_1 MULBERRY VARIETY

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ABSTRACT: *The relationship between the quantity of mulberry leaves ingested and the production of silk is an important aspect in sericulture. The performance of larval stage, depends upon the nutritional value of mulberry leaves it consumes (Morohoshi, 1976; Rapusas and Gabriel, 1976; Koul et.al.1979., Bari et.al. 1985). The larval growth in insects is marked by changes in the body form. Different parts of the body grow at different rates. The Tissue Somatic Index is one of the prime parameters and its application in modern biology has great advantage to understand and interpret the various organ development patterns of an individual against a standard (Venkatarami Reddy and Benchamin, 1989). The factors such as temperature, humidity and photoperiod affect the growth and physiology of silkworm right from hatching to spinning of cocoon. Effect of photoperiods and seasons on development of insects was observed by Danielevskii, 1965.*
The photoperiod changes played vital role on growth pattern of silkworm. Among environmental factors apart from temperature, photoperiods also affect the growth and development of silkworm (Dariuez Piesik, 2006).
Increased larval weight, silkgland weight as well as TSI was observed in both M_5 and V_1 mulberry variety fed larvae when exposed to 18 hrs. of light, while decreased when larvae were exposed to 18 hrs dark.

Keywords: *Bobmbyx mori L, Photoperiod, Tissue somatic index, mulberry varieties.*

I. Introduction

Silkworm *Bombyx mori* L is very commonly used for the commercial production of silk. The output of the silkworm get affected by various environmental factors.Nutritional background of the larval stage significantly influences the status of the resulting pupae, adult and production of silk.The quantity, rate and quality of food consumed by an insect larva have great bearing on its survival, growth rate, development duration and final body weight. A mutual relationship among the food ingested, digested and digestibility in temperate silkworm, *Bombyx mori* L. has been elucidated by Ueda and Suzuki, 1967; Yamamoto and Gamo, 1976. Nutrition involves biochemical and physiological activities which transform food elements in to body elements.The relationship between the quantity of mulberry leaves ingested and the production of silk is an important aspect in sericulture.

Investigation on photoperiodic influence on consumption and utilization of food during different instars and consequently on cocoon characteristics, have been carried out (Shaarawy *et.al.,* 1978, b; and Kastur Bai and Ananthnarayana, 1980). Higher photoperiod seems to induce higher protein biosynthesis in the silk and in the silk gland (Tazima, 1978).The relationship between the quantity of mulberry leaves ingested and the production of silk is an important aspect in sericulture. The performance of larval stage, depends upon the nutritional value of mulberry leaves it consumes (Morohoshi, 1976; Rapusas and Gabriel, 1976; Koul *et.al.*1979., Bari *et.al.* 1985). The Tissue Somatic Index is one of the prime parameters and its application in modern biology has great advantage to understand and interpret the various organ development patterns of an individual against a standard (Venkatarami Reddy and Benchamin, 1989).The photoperiod changes played vital role on growth pattern of silkworm. Among environmental factors apart from temperature, photoperiods also affect the growth and development of silkworm (Dariuez Piesik, 2006).In the present study effect ofdifferent photoperiods on growth pattern was studied.

II. MATERIALS AND METHODS

MATERIAL

The Pure Mysore multivoltine silkworm race of *Bombyx mori* (L.) was selected for the study.Two varieties of mulberry V_1 and M_5 were used to feed the silkworm *Bombyx mori* (L.).

METHODS

Method followed for rearing was by Krishnaswami *et al* 1978.The rearings were carried out at different photoperiods such as, 18 hrs. light: 6 hrs dark and 18 dark: 6 hrs light.

Growth pattern:

(i) Larval duration:

The total larval duration required from the hatching of the larvae to the time of beginning of spinning was recorded. This duration was expressed in days.

(ii) Weight of larva:-

On the fifth day of Vth instar, group of 10 larvae were collected randomly and weighed accurately on electronic balance. The mean was calculated and was expressed in mg / worm.

(iii) Weight of silk gland:-

The weighed larvae on fifth day of Vth instar stage were dissected. The silk glands were isolated and weighed accurately on electronic balance. The mean was calculated and the weight was expressed in mg.

(iv) Tissue Somatic Index (TSI):-

After recording the total silk gland weight and the total body weight of larva, Tissue Somatic Index (TSI) of silk gland was calculated as described by (Vani, 2003). The formula used to calculate TSI is as follows:

$$TSI = \frac{\text{Wt of silk gland (gms)}}{\text{Wt of larva (gms)}} \times 100$$

Food consumption and utilization:-

The larvae were fed on V_1 and M_5 mulberry varieties. The parameters like food consumption and food utilization were calculated by the method of Waldbauer, 1968.

(i) Food consumption (C) = Wt of leaves supplied (gms) – Wt of leaves left over (gms)

(ii) Food Utilization (U) = Food ingested (C) (gms) – Wt of faeces (gms)

III. RESULTS AND DISCUSSION

Perusal of Table No. 1 reveals the results of different photoperiods on growth pattern of silkworm reared on M_5 mulberry variety. When the silkworms were exposed to 18 hrs. of light, weight increased in both larvae and silkgland and there was increase in Tissue Somatic Index also (2.031 gms, 0.357gms and 17.57 respectively). However, when silkworms were exposed to 18 hrs. dark, weights of larvae and silkgland as well as TSI decreased (1.77gms and 0.272gms and 15.36 respectively) as compared to the control (1.673 gms, 0.256gms and 15.30 respectively).

Same pattern of results was obtained in case of V_1 mulberry variety fed larvae and different photoperiods on growth pattern of silkworm. The weight of larvae and silkgland, and Tissue Somatic Index increased significantly when exposed to 18L:6D Photoperiod (2.582gms, 0.491gms and 19.01 respectively). When the larvae were exposed to 18 hrs. dark there was increase in case of larval weight (2.135gms) while decrease in case of Tissue Somatic Index (15.64), in comparison to control (1.939 gms and 16.70 respectively). Weight of silkgland (0.334gms) remains at par with control (0.324gms) (Table No. 2).

Table 1. Effect of different photoperiods on growth pattern of silkworm *Bombyx mori* (L.) reared on M_5 mulberry variety.

Sr. No.		Weight at different photoperiods (gms)		
		Control	18L:6D	18D:6L
1.	Larvae	1.673 (±0.061)	2.031*** (±0.028)	1.77 (±0.057)
2.	Silk gland	0.256 (±0.015)	0.357 (±0.090)	0.272 (±0.014)
3.	TSI	15.30 (±0.26)	17.57* (±0.66)	15.36 (±0.90)

Figures in parenthesis indicate standard deviation

* P < 0.05*** P < 0.001

Table 2. Effect of different photoperiods on growth pattern of silkworm *Bombyx mori* (L.) reared onV_1 mulberry variety.

Sr. No.		Weight at different photoperiods(gms)		
		Control	18L:6D	18D:6L
1.	Larva	1.939 (±0.055)	2.582 (±0.16)	2.135 (±0.020)
2.	Silkgland	0.324 (±0.0085)	0.491 (±0.028)	0.334 (±0.008)
3.	TSI	16.70 (±0.75)	19.01* (±0.70)	15.64 (±0.59)

Figures in parenthesis indicate standard deviation
* P < 0.05

Figures in Table No. 3 indicate the results obtained for food consumption and utilization of silkworm larvae fed on M_5 mulberry variety at different photoperiods. It increased significantly when exposed to 18L:6D photoperiod (7.891gms and 5.029gms respectively) while it was at par in case of 18 hrs dark (7.534gms and 3.994gms respectively) in comparison to the control (7.586gms and 3.882gms respectively).

Same pattern observed in case of V_1 mulberry variety fed larvae at different photoperiods in case of food consumption and utilization. There was significant increase in both cases when exposed to 18 hrs light (10.233gms and 5.741gms respectively) and decreased when exposed to 18 hrs. dark (8.969 gms and 4.852 gms respectively) as compared to the control (8.485gms and 4.844gms respectively) (Table No. 4).

Table 3. Effect of different photoperiods on food consumption and utilization of silkworm *Bombyx mori* (L.) reared on M_5 mulberry variety.

Sr. No.	Component	Weight at different photoperiods (gms)		
		Control	18L:6D	18D:6L
1.	Food consumption	7.586 (±0.10)	7.891 (±0.39)	7.534 (±0.34)
2.	Food utilization	3.882 (±0.36)	5.029 ** (± 0.45)	3.994 (±0.75)

Figures in parenthesis indicate standard deviation
** P < 0.01

Table 4. Effect of different photoperiods on food consumption and utilization of silkworm *Bombyx mori* (L.) reared on V_1 mulberry variety.

Sr. No.	Component	Weight at different photoperiods (gms)		
		Control	18L:6D	18D:6L
1.	Food consumption	8.485 (±0.34)	10.233*** (±0.389)	8.969 (±0.73)
2.	Food utilization	4.844 (±0.450)	5.741* (±0.54)	4.852 (±0.35)

Figures in parenthesis indicate standard deviation
*** P < 0.001

Among environmental factors apart from temperature, photoperiods also affect the growth and development of silkworm (Dariuez Piesik, 2006).

Increased larval weight, silkgland weight as well as TSI was observed in both M_5 and V_1 mulberry variety fed larvae when exposed to 18 hrs. of light, while decreased when larvae were exposed to 18 hrs dark.

Various factors such as temperature, humidity, photoperiod etc affect the food consumption and utilization also when the larvae were exposed to 18 hrs. light the food consumption and utilization

increased significantly in both the cases of M_5 and V_1 mulberry variety fed larvae, while decreased when exposed to 18 hrs. dark.

It can be concluded that if the silkworm are exposed to 18 hrs light it may result in better yield and quality of silk because it shows significant increase in growth rate while fed onM_5 and V_1 mulberry variety.

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OCCURENCE OF ANTS IN IRRIGATED RICE FROM WESTERN MAHARASHTRA, INDIA

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ABSTRACT: *Ants are economically important cosmopolitan insects. These have much economical importance including role as pests, bio-control agent and food for other animals. Diversity of ants from rice field is less explored from western Maharashtra. Hence, survey of ants from paddy field was performed to understand the diversity of ants. Overall 22 species of ants belonging to 5 subfamilies and 16 genera were recorded in the paddy field. Tapinoma sp. and Pheidole spp. were common while Solenopsis geminata was found abundant. Tapinoma sp. was aggressive and S. geminata was foraging on rice. The study may be helpful in management of ants for its utilization in bio-control and to control the pest ants in the rice field.*

Keywords: *Ants, economic importance, rice field, western Maharashtra.*

I. Introduction

Ants are significant part of ecosystem and also act as ecosystem engineers. Ants have a major influence on other organisms in many habitats including agricultural fields where some predatory species are important as biological control agents (Carroll and Risch, 1983). However, knowledge of their occurrence and predatory activity in rice fields seems limited to casual observations. These were not identified to species level previously from Western Ghats of Maharashtra. Occurrence of major dominant species is also required to utilize the ants for biological control. *Solenopsis geminata* the fire ant, prey on eggs of the rice bugs. *Camponotus, Monomorium, Pheidole, Crematogaster spp*. have been observed preying on eggs and larvae of the rice leaf folder (Van Zwalunwenburg (1928); Miller (1930); Rawat and Diwakar (1982). As a basis for studies of the role of ants in biological control of rice pests, this paper reports different species of ants from irrigated rice fields.

II. MATERIALS AND METHODS

Western Maharashtra is a hilly region and most of agricultural land used for rice cultivation. Study was conducted in five different rice farms for monthly observations. Most work was done in irrigated rice fields of Western Maharashtra namely Sangli, Satara and Kolhapur districts. The rice fields planted with a wet and dry season of each year. During the survey, the ants were collected from 2017-2018 at fifteen days of intervals, generally in morning and evening from six different localities of Western Maharashtra. Collection of ants was done by sweeping the insect sweep net and by hand picking method with the help of camel hair brush and forceps. Collected ants were preserved in the collection bottle field with 70% alcohol. Ants were identified with the help of identification key of Bolton (1994), The Fauna of British India (Bingham, 1903).

III. RESULTS AND DISCUSSION

Present study reports 22 species of ants found in the irrigated rice field of Western Maharashtra. The all 22 species are belonging to 16 genera and 5 subfamilies of Family Formicidae (Table 1). The largest subfamily found was Myrmicinae with 10 species followed by Formicinae, Dolichoderinae, Penerinae and Cerapachyinae in study area (Fig. 1). The genus *Monomorium* was found dominant with four species followed by *Crematogaster* (2), *Camponotus* (2) and *Pheidole* (2). Other genera represented only one species each (Fig. 2). Four genera out of 16 were found dominant in the study area.

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Figure 1. Subfamily wise species composition of family Formicidae.

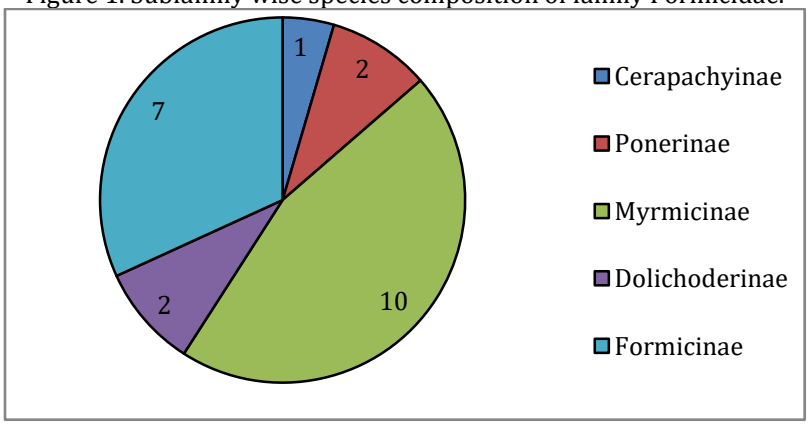

Figure 2. Species composition of Genera of Family Formicidae.

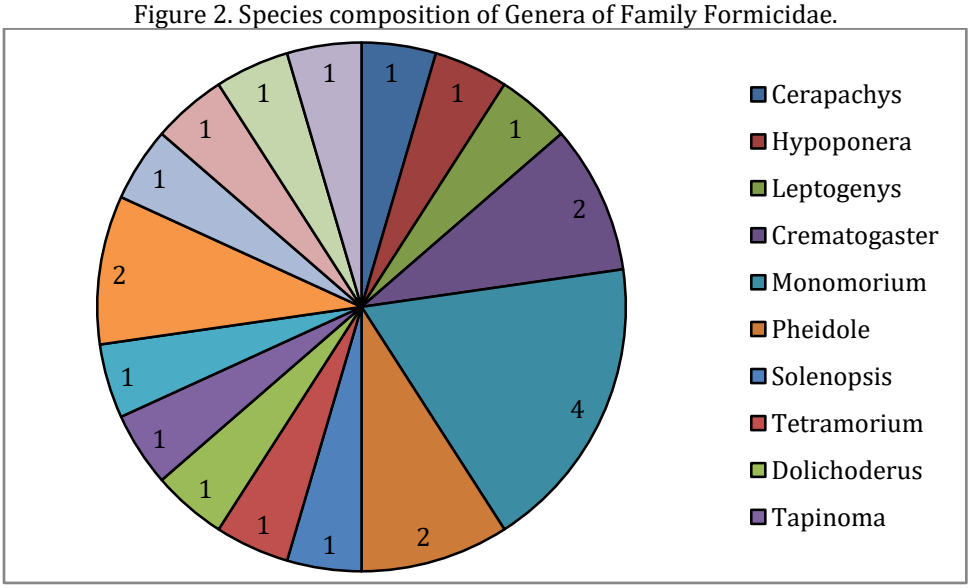

Table 1. Different species collected from the irrigated rice fields in Western Maharashtra

Subfamilies	Genera	Species
1) Cerapachyinae	*Cerapachys* Forel, 1893	*Cerapachys rufus* (Jredon)
2) Ponerinae	*Hypoponera* Jerdon,1851	*Hypoponera sp.*
	Leptogenys Roger 1861	*Leptogenys chinensis* (Mayr)
3) Myrmicinae	*Crematogaster* Lund,1831	*Crematogaster wroughtoni (Forel)*
		Crematogaster sp.
	Monomorium Mayr,1855	*Monomorium destructor* (Jredon)
		M. Monomorium Bolton
		M. pharaonis L.
		Monomorium sp.
	Pheidole Westwood, 1841	*Pheidole spp. 2*
		P. diversus (Jerdon)
	Solenopsis Westwood, 1841	*Solenopis geminate* Fabricius
	Tetramorium Mayr,1855	*Tetramorium smithi* Mayr
4) Dolichoderinae	*Dolichoderus*	*Dolichoderus affinis* Emery

	Tapinoma Forester 1850	*Tapinoma melanocephalum* Fabricius
5) Formicinae	*Anoplolepis*	*Anoplolepis gracilipes* Smith
	Camponotus Mayr, 1861	*Camponotus compressus* Fabricius
		Camponotus variegates. Smith
	Oecehylla Smith, 1861	*Oecehylla samaragdina (Fabricious)*
	*Paratrechina*Motschoulskys1863	*Paratrechina longicornis* Latreille
	Plagiolepsis Mayr,1861	*Plagiolepsis jerdonii* (Forel)
	Polyrachis Smith,1858	*Polyrachis dives* Smith

IV. DISCUSSION

In view, the knowledge of ants in irrigated rice fields, this work was essential for role in biological control of rice pests. A wide range of ant- plant interactions has been published (Huxley and Cutler, 1991).

In these circumstances and particularly where small patches of rice are grown amongst dry land tree vegetation. Many species of widely foraging ants may prey on insects in rice fields (Way and Khoo, 1992). *S. geminata* observed abundance in rice fields.

We observed the heavy occurrence of ants in Western Maharashtra namely, Sangli, Satara and Kolhapur districts. About 22 species of ants from 16 genera have been observed from this region. This study contributes to rice pest control and beneficial ants which are natural enemies.

V. CONCLUSION

The North Western Maharashtra has very good diversity of ants in the rice filed. There were 22 species of ants belonging to 5 subfamily and 16 genera of family Formicidae reported. Most diverse subfamily was Myrmicinae and the most diverse genus Monomorium reported from the rice field. The species *Solanopsis* was found abundant and most common.

VI. ACKNOWLEDGEMENT

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Fig.1. ***S. geminata***

Fig.2.*Tapinoma melanocephalum*

Fig.3. ***P. megacephala***

Fig.**4.** ***Polyrachis sp.***

Fig.5.*Crematogaster sp*

Fig.6. ***C. compressus***

Fig.7.*Camponotus variegatus*

Fig.8. ***O. smaragdina***

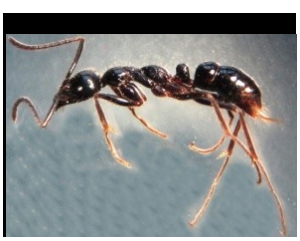

Fig.9. ***Leptogenys chinensis***

HISTOPATHOLOGICAL ASSESSMENT OFUROLITHIASIS IN RODENTS: EXPERIMENTAL MODELS AS *RATTUS NORVIGICUS*

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ABSTRACT: *Urolithiasis (nephrolithiasis) relates with kidney stone disease. It get developed to urological disorder, associated with an increased risk of end-stage renal failure. Several physicochemical events including supersaturation, aggregation, crystallization nucleation, growth and retention of urinary stone constituents within renal tubular cells. Understanding of the pathophysiology of nephrocytes with altered blood and urine content found thrust area of urolithiasis; therefore, present investigation has intended to provide focus on information regarding kidney stone pathogenesis, and biochemical changes. Vertebrate model rat have been used to understand histomorphological and stone pathogenesis. Chronic dose of Ethylene glycol (EG) can cause nephritic toxicity by the formation of calcium oxalate crystals. For the different concentration and exposure period nephrocytes were examined histologically and interpreted for any alterations in rate of urine filtration and content against experimental model Rattus norvigicus.*

Keywords: *Urolithiasis, Ethylene glycol (EG), Stone induction, Histology, Rattus norvigicus.*

I. Introduction

Renal stone is a common disease, occurring in 8% of the population. This disease is multifactorial and found related to environmental factors, especially western diet. However, while the ingestion of oxalate-rich food is easily preventable, other urolithiasis-associated factors are more or less veiled and include various genetic mutations that alter general metabolism and homeostasis.

In the pathophysiological studies numbers of species have been evaluated against variety of toxic chemicals. Although considerable variability has been observed in sensitivity across species, strains and even sexes of animals. Now a day's no single animal model system can be thought of as perfect, whereas among the rodents rats proved to be well-established, relatively economical model that scientists have recommended. Meneton, et al., (2000) described major morphological and physiological specifications in renal physiology of rodents especially, mouse. Khan et., al. (2002a) and (2013) documented that, different animal models have varied capacity of bioconcentration of calcium oxalate in different regions of nephritic cells responsible for kidney stone formation. Worcester et., al.,(2005), also noted rat as a animal model for top study of hyperoxaluria and nephrolithiasis with related assessment in small bowel resection. The crucial moment in the pathophysiology of kidney stones is the formation of crystals in the tubular fluid or urine. For the relevant studies, calcium phosphate supersaturation regulating mechanism for regulates stone formation was noted by (Bushinsky et., al. 2000). Pottenger et., al. (2001) concluded physiological alterations of dose-dependent nonlinear pharmacokinetics of ethylene glycol metabolites in pregnant and nonpregnant Sprague-Dawley rats by applying oral administration of ethylene glycol. Whereas Evan, et al. (2004) localized Calcium oxalate crystal by applying immunostaining in genetic hypercalciuric stone-forming rats. Rule et. al., (2010) have critically analyzed impact of nephritic calculus and its relevant pathologies for changing physiological functioning of circulatory and cardiac infraction. Carney et. al., (2001) documented dose dependent cellular changes in the developmental physiology of animals. Khan and Glenton, (2010) explained induction protocol of calcium oxalate in relation to the severity for the development of nephrolithiasis in mice. Aggarwal et. al.,(2013) given molecular mechanism of renal stone formation and the critical role played by modulators. Mikawlrawng and Vandana, (2014) documented effect of medicinal plants as antiurolithiatic agents in the experimental animals and recorded minimized level of nephritic crystallization after the therapeutic dose induction. Viers ,et., al. (2015) also critically assessed endoscopic and histological findings in a cohort of uric acid and calcium oxalate stone formers and concluded the pathological report on the vital organs as kidney.

By considering the available documentation and with well recommended, relatively economical model as rat, the present work has been carried out to understand pathophysiology of urinary tract with chronic impact of EG on the development of calculus and crystallization with physiological nephritic disturbances in Rattus norvigicus.

II. MATERIALS AND METHODS

For the present investigation, female wistar rats Rattus norvigicus were used. The investigation was carried out with permission of authorized CPCSEA approval for animal experiment. Animals were housed individually in mesh cages. Animals were maintained in accordance with the guidelines as per the care and use of laboratory animals. Following standard protocol, animals were reared and breed in animal house of Department of Zoology, Shivaji University, Kolhapur. Mean Daily animal room temperature ranged from 710F to 720F (21.90C to 22.40C) with mean daily relative humidity ranged from 38% to 50% during the study. During the experiment animals were exposed to 12-hour light/12-hour dark photoperiod. Animals were fed on regular remanded diet ad libitum. Reverse osmosis–treated water was provided to ad libitum from water bottles as per need.

All the experimental animals were under observation daily and detailed records were made systematically. During experiment each of the animal was weighed before and after the treatment with Ethylene glycol (EG) . Extensive urine analyses had been conducted in the prior studies where, urine samples were collected over an approximately 24-hr. period from all animals during morning 09.00 to 10.00 am. without any preservatives for analysis. Collected samples were visually observed and evaluated for color, total volume, appearance and microscopic sedimental analysis. In urine analysis, 1 ml of the fresh urine sample was centrifuged at 3000 rpm (revolutions per minute) for 10 minutes, then supernatant was discarded. The type and number of the crystals were photographed, identified and counted using Inverted Phase Contrast microscope. The severity of supersaturation and crystallization was observed and graded and documented as per the dose and exposure periods.

For histopathological study, experimental rats were sacrificed after the induced exposure period as per the experimental protocol, blood samples (01 to 02 ml) was subjected for biochemical analysis. Interested and targeted both kidneys were harvested and morphometrically observed. Nephritic tissue was subjected to standered micrtechnique by fixing it into 10% buffered formalin, embedded in paraffin, section were cut to 5-6 µ m section, sections on slides were stained with Hematoxylin and Eosin technique. The slides were examined under Inverted Phase Contrast microscope for the histopathological observation. The obtained results were presented in the form of tables, graphs and photo plates. Compiled data was interpreted for toxicity study and Urolithiasis.

III. RESULTS AND DISCUSSION

Over the past decade, major advances have been made in understanding the pathogenesis, diagnosis and also about treatment of kidney stone disease. Giannossi and Summa (2012), provided number of technical methods for pathological biomineral analysis and classification schemes regarding the toxicological study. Urolithiasis is a multifaceted process that initiates with the formation of microcrystals. Various factors may be involved in the pathogenesis of urolithiasis in model and the disease. Ethylene glycol administration is a common method for the experimental induction of urolithiasis in rats. Results of the study were as follows-

a) Noticeable alterations:

During the experiment we found that, controlled group animals were active and healthy throughout the study period. Overall their body weights were found increased. The daily water consumption was average 31 to 32 ml/ day, which was found related to the rate of urine synthesis. Average volume collected was 9 to 10 ml. In the blood assessment we found that the level of blood calcium was elevated wit induction of EG and exposure period confirming altered metabolism of calcification and concentration (Table: No. 1 and Graph No. 1). In comparison, after the induction of EG for 15 day and 30 days, of period, we noticed that, water consumption rate and the rate of urine formation was remarkably reduced leading to showing enhanced mechanism of crystal formation. Pathological examination of kidney tissue showed that, all rats in Groups 2 and 3 had crystal deposited in their kidney. The crystal density in Group 3 (1.0%) was slightly higher than that of Group 2 (0.5%). Most studied mechanism for the formation of calcium stones was increased with urinary supersaturation of stone-forming salts. Agrawal, et. al.,
(2013) proved mechanism of Calcium oxalate supersaturation in the experimental model and documented nephritic damage. Tsuji et., al. (2014) noticed stepwise development of hyperoxaluric condition in rats resulting to renal calcium oxalate crystal deposition. As pathic condition it leads to homogeneous nucleation in the lumen of the nephron, followed by crystal growth and consequent obstruction in the distal nephron. We observed some correlation between size of nephritic cells and the presence of kidney crystal deposits (Plate No. 1). No rats died during the course of the study period.

b) Histopathology of Kidney tissue :

After subjecting the tissue for 15 and 30 days induced exposure, histopathological sections showed tubular distruction and damaged integrity of the epithelial tubule in the interim part of the nephrocytes as compared to control section (Plate No.2).

Under the microscope we found that, some cells have been injured with cellular nucleation and has developed nidus which has promoted the retention of particles on the renal papillary surface. Wiessner et al., (2011) pointed out scientific methodology for accuretely inducing the hydroxyproline for the development of hyperoxaluria. McMartin (2009) , documented appearance of calcium oxalate crystals resulting to severe damage to kidney and renal failure against ethylene glycol poisoning. Similarly, Corley et., al. (2002) recorded, ethylene glycol induced damage in nephron of pregnant rats showed continuous subcutaneous infusion in the targeted tissues. Daudon (2004), microscopically valued crystalluria and quantitative morpho constitutional anomalies in urinary calculi leading to sever damage. Khan, et al.,(2016) complied the overall impact of Kidney stones formation with pathetic conditions obtained by inductions of different chemicals.

In support to this, mechanism of nephrolithiasis and disturbed function of kidney was well documented by (Sigurjonsdottir et., al. 2015). In continuation to this, Sofia et. al.,(2016) documented some prevalence and risk factors regarding kidney stone and its ill effects. Afsar et al.,(2016), recorded biochemical mechanism of sodium intake in nephrolithiasis and its epidemiology with degenerative morphology of kidney. Despite considerable improvements many aspects of renal stone formation remain unclear. However, as per the literature, renal cell injury, crystal retention, cell apoptosis, Randall's plaque, and associated stone inhibitors or promoters play important roles for kidney stone formation. As in nutshell, in the present experiment, animal kidneys showed alterations as metabolically calcium oxalate is capable of destruction and damage of the apical membrane of proximal tubule cells and impairing urine flow by decreasing kidney functions. Quantitatively and/or qualitatively, decreased volume and supersaturation of calcium resulted in renal stone formation or crystal deposition and excretory pathology.

Table No- 1. Control Group :

No. of Day	Water Consumed (ml)	Volume of Urine (ml)	Blood Calcium mg/dl
Day 1st	35.6 ±1	9.0 ±1	9.60 ±1
Day 15th	31.1 ±1	9.4 ±1	9.60 ±1
Day 30th	34.3 ±1	8.9 ±1	9.60 ±1

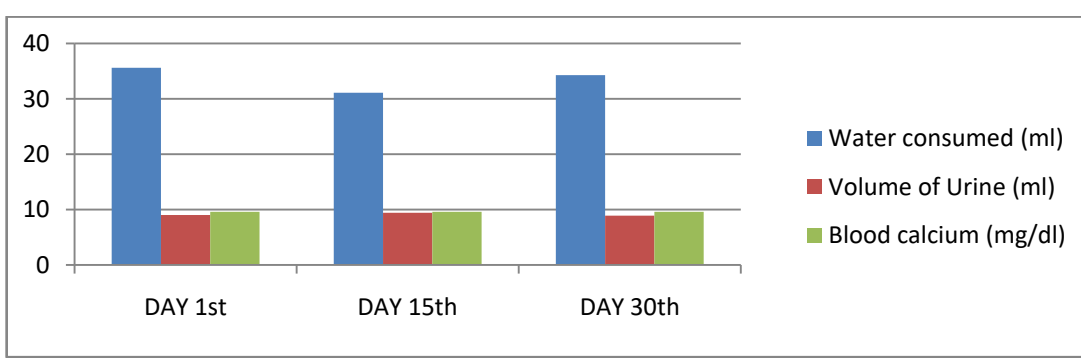

Table No-2. .5% Ethylene Glycol Induced Rat

No. of Day	0.5% EG Dose Consumed (ml)	Concentration of Dose (µl)	Volume of Urine (ml)	Blood Calcium
Day 1st	23.2 ±1	115.575 ±1	8.7 l ±1	9.60mg/dl
Day 15th	26.5 ±1	130.65 ±1	7.63 ±1	-
Day 30th	23.4 ±1	115.55 ±1	5.54 ±1	9.58 mg/dl

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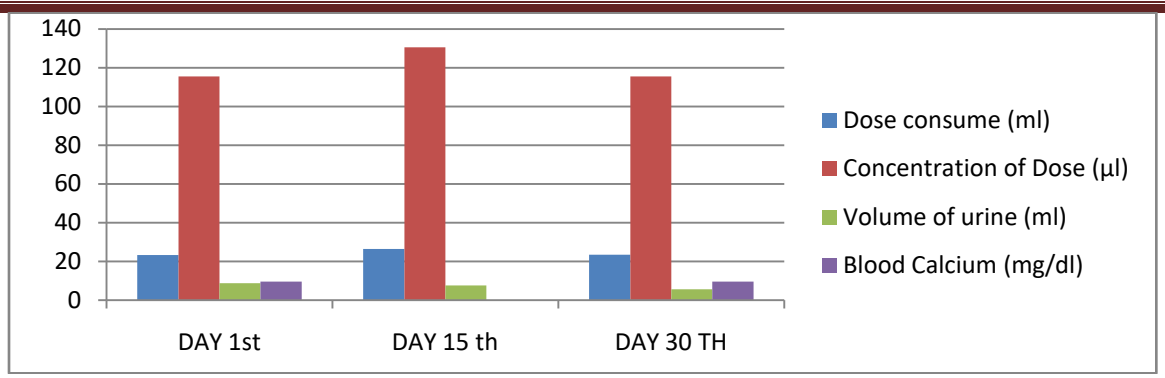


Table No -3. 1 %Ethylene Glycol Induced Rat

No. of Day	1% EG Dose Consumed (ml)	Concentration of Dose (µl)	Volume of Urine (ml)	Blood Calcium
Day 1st	20.1 ±1	202.02 ±1	9.2 ±1	9.60 mg/dl
Day 15th	24.3 ±1	245.424 ±1	6.6 ±1	-
Day 30th	28.4 ±1	282.828 ±1	4.1 ±1	10.20 mg/dl

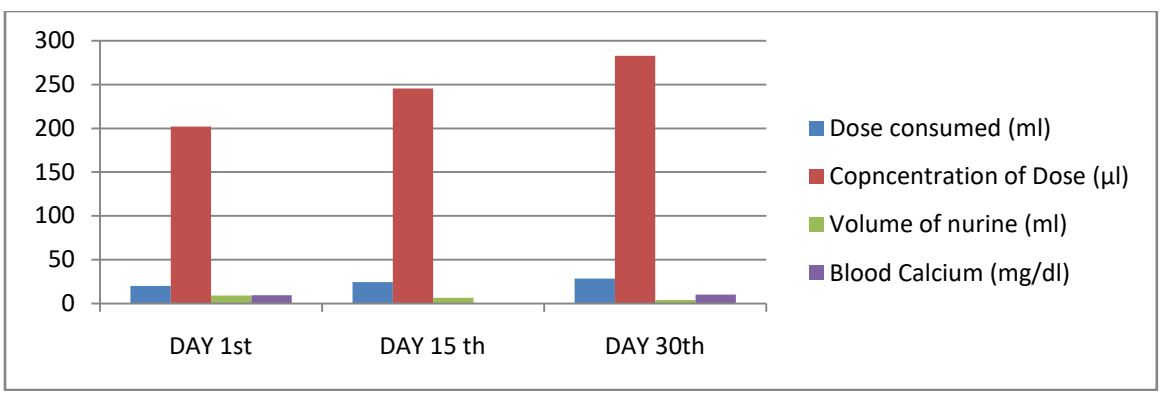
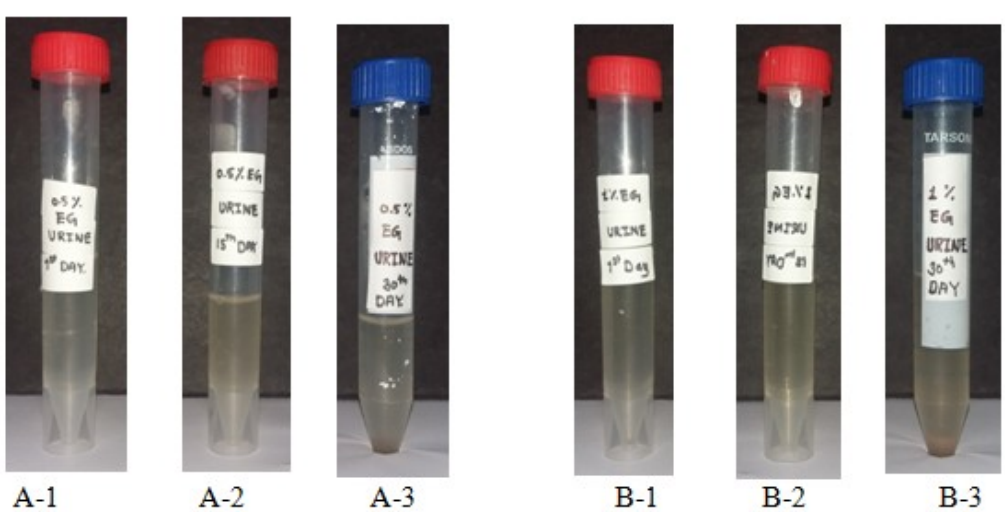
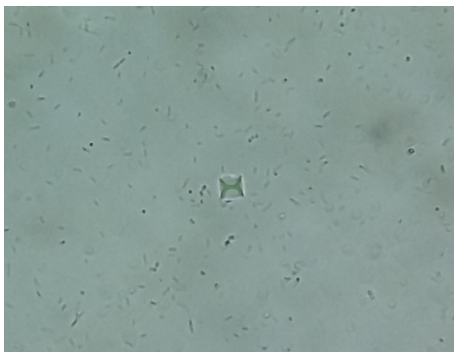
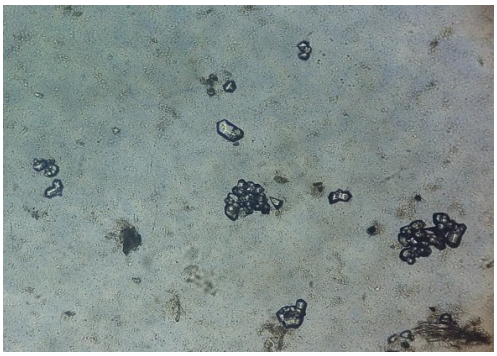


Plate No. 1





A 4



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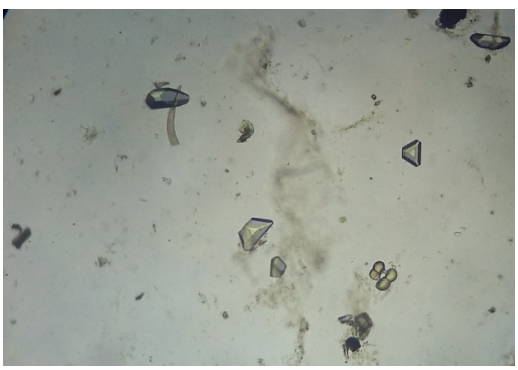
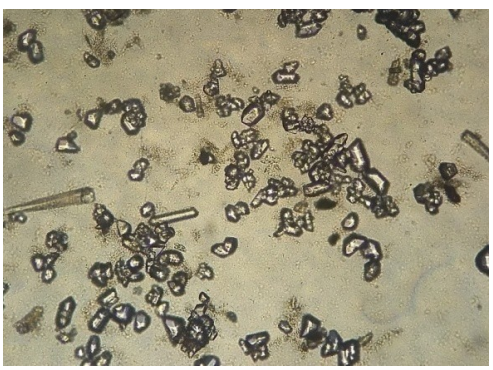


A 6



A 7

Plate No.2

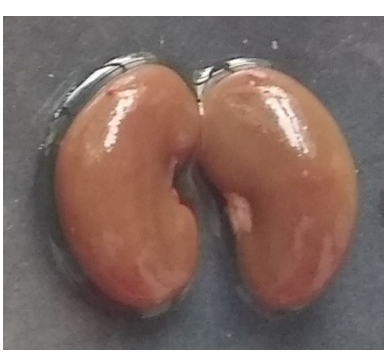


Figure A

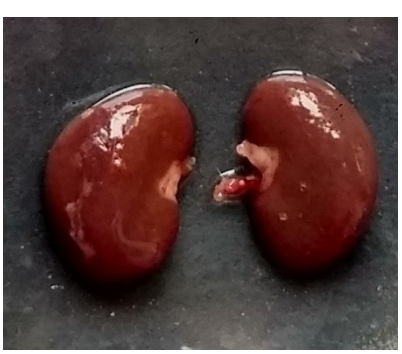


Figure B

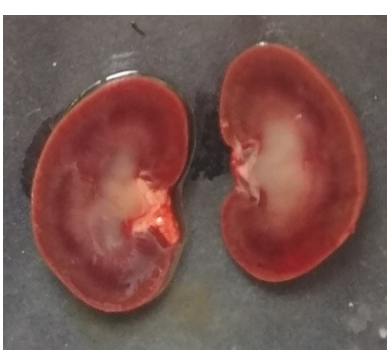


Figure C

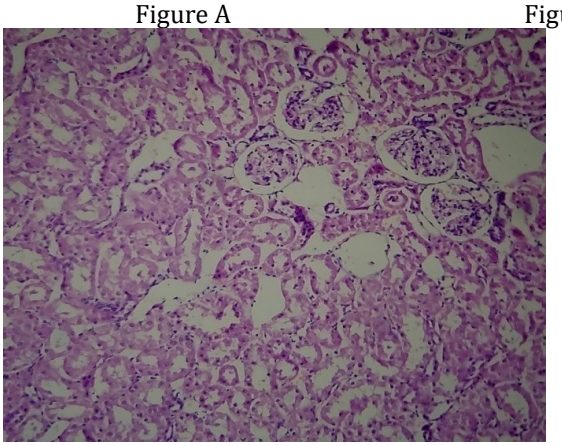


Figure D

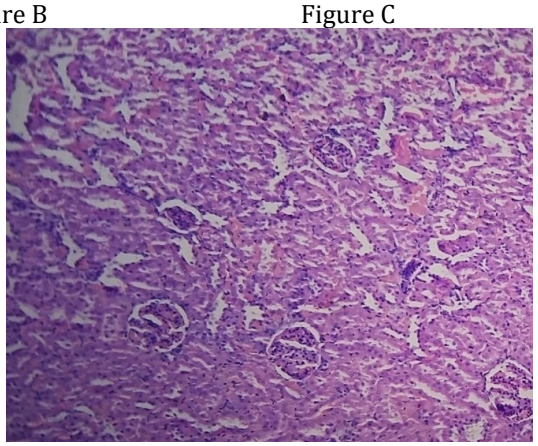


Figure E

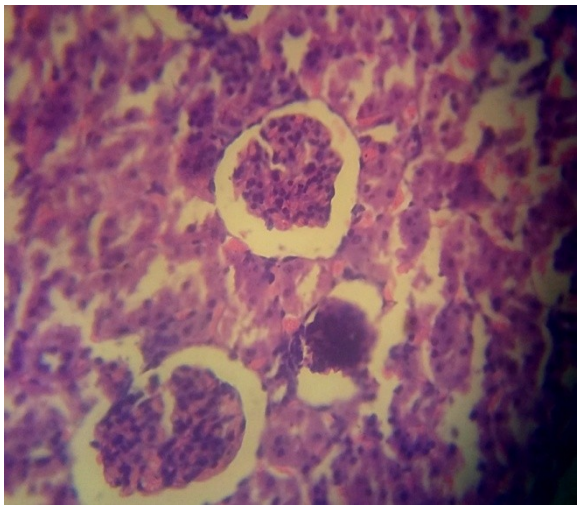


Figure F

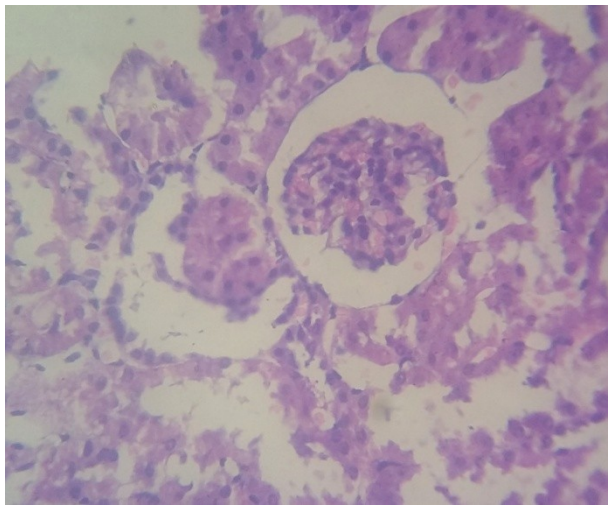


Figure G

Plate No. 1- A1- 0.5% EG urine- 1 st day collection, A2- 0.5% EG URINE 15TH day collection. A3- 0.5% EG urine 30 th day collection. A4- 0.5% EG urine Sample 10x microscopic observation 15th day. A5- 0.5% EG urine sample 10 x microscopic observation 30th day. B1- 1% EG urine 1 st day collection, B2- 1% EG URINE 15TH day collection.
B3- 1% EG urine 30 th day collection, B4- 1% EG urine Sample 10x microscopic observation 15th day, B5- 1% EG urine sample 10 x microscopic observation 30th day.
Plate No. 2 figure- a 0.5% EG kidney 30 days, figure- b 1% EG kidney 30 days
figure- c ls of 1% kidney , figure- d 0.5% EG 30 days kidney ls under 10x magnificationfigure- e 1% EG 30 days kidney ls under 10x magnifiaction
figure- f 0.5% EG 30 days kidney ls under 40x magnifiaction
figure- g 1% EG 30 days kidney ls under 40x magmnifiaction

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ANTIOXIDANT ENZYME STATUS OF CYSTEAMINE –HCL INDUCED DUODENAL ULCER IN AGED MICE AFTER *ALOE VERA GEL* TREATMENT

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ABSTRACT: *Peptic ulcer (gastric/duodenal) is one of the most common gastrointestinal (GIT) disorders and has affected humans for centuries. In the present investigation we have studied antioxidant enzyme activity of duodenum of cysteamine induced duodenal ulcerated aged mice after Aloe vera gel treatment. Present findings exhibited that decreased level of SOD, CAT, GSH and increased LPO concentration which was restored by the administration of Aloe vera gel in both male and female old mice. Aloe vera gel supplementation by its antiulcer and antioxidant properties lowers the oxygen free radicals implicated in the pathogenesis of ulcer ultimately reduces the duodenal muocosal erosion and restores the antioxidant enzyme level in both sexes of older mice.*

Keywords: *Cyteamine – HCl, antioxidants, Aloe vera gel , duodenal ulcer, aged mice*

I. Introduction

Peptic ulcer (gastric/duodenal) is one of the most common gastriointesinal (GIT) disorders and has affected humans for centuries over the worldwide. A peptic ulcer is necrotic changes occurred in the inner surface of the stomach or duodenum, resulting in loss of tissue and inflammation. The disturbed or damaged protective lining of the stomach and duodenum may be superficial or become deeply erosive (Sivri, 2004) results into altered offensive and defensive mechanism of mucosal linings. Both type of ulcer develops as a due to imbalance between mucosal defensive (protective) factors such as mucosal blood flow, ischemic preconditioning, NO and PG generation, growth factors, ghrelin, and aggressive factors such as HCl, pepsin, bile acids and others (Tanigawa, et al.,2005; Noor, et al.,2006).Different factors are reposible for the formation of ulcer include prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), Helicobactor pylori (H. pylori) infection, stress, alcohol, smoking (McGuigan, 1991) and spicy food.The rate of acid secretion may be increased seen in pathogenesis of duodenal ulcer (Marshall, 1995; Wormsley, 1997).Different drugs induced ulcerated animal models are widely used to study the pathogenesis of ulcer as well as to evaluate the ulcer curative properties of different medicinal plants. Cysteamine or propionitrile induced duodenal ulcer, acts an excellent model for duodenal ulcer formation (Selye and Szabo, 1973; and Adler, et al., 1983). Cysteamine- HCl alters the structure and secretary function of Brunner gland via cellular exfoliation and erosion in female mice (Ashokan, et al., 2010).Number of the morphological, histological changes and imbalanced biochemical secretary activity of mucosal lining of duodenum leads to erosion during development of cysteamine induced duodenal ulcer reported by Poulsen,et al.,1981 and Szabo and Reichlin, 1989. Oxidative stress that is formation of ROS (Reactive oxygen Species) implicated in the pathogenesis of ulcer (Choi, et al.,2012). The ROS generated by the impaired metabolism may contribute to gastric mucosal damage. (Kwiecien, et al., 2002 and Nasuti, et al., 2006). Duodenal ulcer formation sensitivity increases with progression of age (Ostensen, et al., 1985 and Crstensen, et al., 2006).

Different medicinal properties bearing plants showing antioxidants cell protective activities via strengthen the functional properties of cells have been concern with various gastrointestinal tract disorders. Aloe vera is medicinal plant belongs to Asphodelacae (Lilliacae) family and it a shrubby or arboescent, perennial, xerophytic, succulent, plant. It is renowned because of its different biological activities (Radha and Laxmipriya, 2015). Aloe vera rich in many phytoconstituents (Reynolds, 2004) include high amount of phenolic compounds, anthrquionone, polysaccharides, alkaloids, polyphenols, aloines (glycosides), aloe emodin.steroids and flavonoids. Glycosides and flavonoids acts as strong antioxidant agents (Tripathi ,et al.,1996) that contributes in the antiulcer (Prabjone, et al., 2006) antioxidant and antidibetic activity (Rajsekaran, et al., 2004) of Aloe vera. The present work performed to demonstrate ulcer curative mechanism and antioxidant properties of Aloe vera gel via assessing the duodenal antioxidants status.

II. MATERIALS AND METHODS

Animals:

Healthy Swiss strain albino mice, *Mus musculus* were used for the present investigation.The breeding pairs were obtained from (Rajarambapu college of Pharmacy, Kasegaon, 209/CPCSEA). Old mice of

16 to 18 month age, weighing 45 to 50 ± 2 gm body weight were used for present investigation. All animals were reared in air-conditioned departmental animal house. They were received Amrut mice feed (Pranav Agro Industries, Pvt. Ltd, Sangli) and water *ad libitum*. Body weight of control group and experimental group were recorded time to time.

Experimental Groups:

Mice were divided into three groups containing six animlas in each group:

Control group: The old mice were given oral administration of 0.5 ml distilled water/ day/ animal for 15 days.

Duodenal ulcer induced group: The old mice was given subcutaneous injection of cysteamine – HCl (40mg/100gm/BW) dissolved in 0.5 ml distilled water (Szabo, 1978).

***Aloe vera* gel treated group:** Both duodenal ulcer induced male and female old mice were given oral administration of *Aloe vera* gel 200 mg/kg dissolved in 0.5ml distilled water/ day/ mouse for 15 days (Subramanian, *et al*., 2007).

After completion of the treatment control, cysteamine HCl administered and *Aloe vera* gel treated animals were weighed and sacrificed by cervical dislocation.The duodenum were removed, weighed and were proceed for SOD(Beaucham and Fridovich 1971), CAT(Luck, 1974), GSH(Ellman's,1986) and LPO (Wills,1966) (lipid peroxidation), antioxidant enzyme concentration studies.

Statistical analysis

All values were expressed as mean ± S.D. The statistical analysis was performed using student's ' t ' test. A value of P<0.001 was considered statistically highly significant.

III. RESULTS AND DISCUSSION

The following graph 1, 2, 3 and 4 shows the SOD, CAT, GSH and LPO (lipid peroxidation), concentration of duodenal tissue in mice from control, duodenal ulcerated and *Aloe vera* treated group respectively.

The graph 1 showed mean concentration of SOD(unit/mg protein/hr) in the control group male and female old mice was evaluated to be 54.88 ± 0.5417 and 52.90 ± 0.9618 whereas it was decreased to 17.54 ± 0.8388 and 20.30 ± 2.683 in duodenal ulcerated group (1:2, P<0.001). After the treatment of *A.vera* gel (200 mg/kg body weight) the SOD concentration was found significantly increased to 33.44 ± 0.6542 and 33.80 ± 1.753 respectively (2:3, P< 0.001).

The graph 2 represented mean level of CAT (unit/mg protein/hr) in the control group male and female old mice was evaluated to be 51.86 ± 1.1480 and 49.48 ± 0.9094 whereas it was reduced 18.98 ± 0.6943 and 17.5 ± 0.9354 in duodenal ulcerated group (1:2, P<0.001). After the treatment of *A.vera* gel (200 mg/kg body weight) the CAT level was found significantly increased to 24.31 ± 0.6395 and 25.26 ± 0.9940 respectively (2:3, P< 0.001).

The graph 3 exhibited mean concentration of GSH (µ/g of tissue) in the control group male and female old mice was evaluated to be 51.86 ± 1.1480 and 49.48 ± 0.9094 whereas it was decreased to 18.98 ± 0.6943 and 17.5 ± 0.9354 in duodenal ulcerated group (1:2, P<0.001). After the treatment of *A.vera* gel (200 mg/kg body weight) the GSH concentration was found significantly increased to 24.31 ± 0.6395 and 25.26 ± 0.9940 respectively (2:3, P< 0.001).

The graph 4 showed mean concentration of LPO (n mol MDA/ mg wet tissue) in the control group male and female old mice was evaluated to be 35.534 ± 1.5589 and 33.164 ± 0.750 whereas it was increased to 49.142 ± 2.0521 and 44.990 ± 1.081 in duodenal ulcerated group (1:2, P<0.001). After the treatment of *A.vera* gel (200 mg/kg body weight) the LPO concentration was found significantly decreased to 39.920 ± 0.7454 and 38.418±1.3255 respectively (2:3, P< 0.001).

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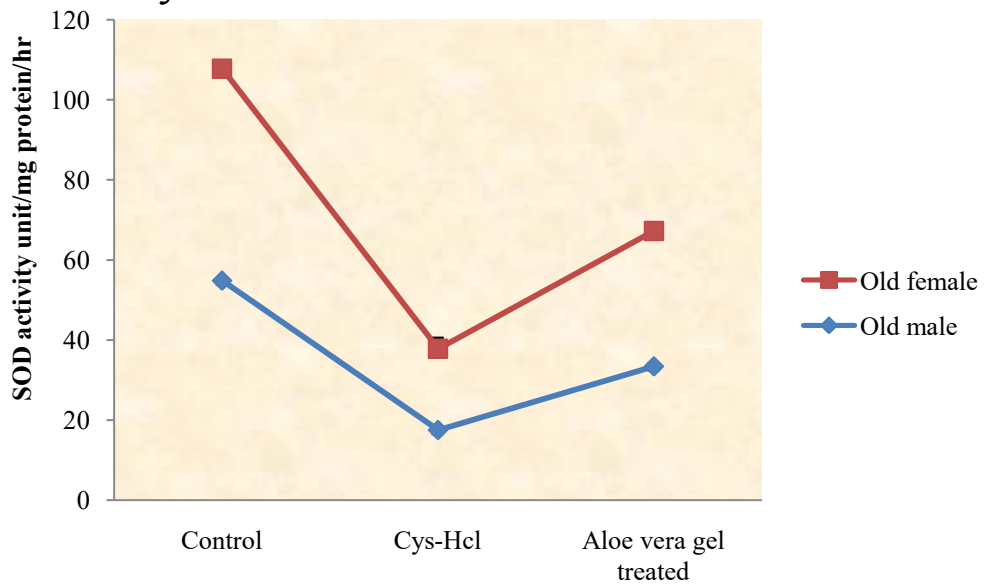
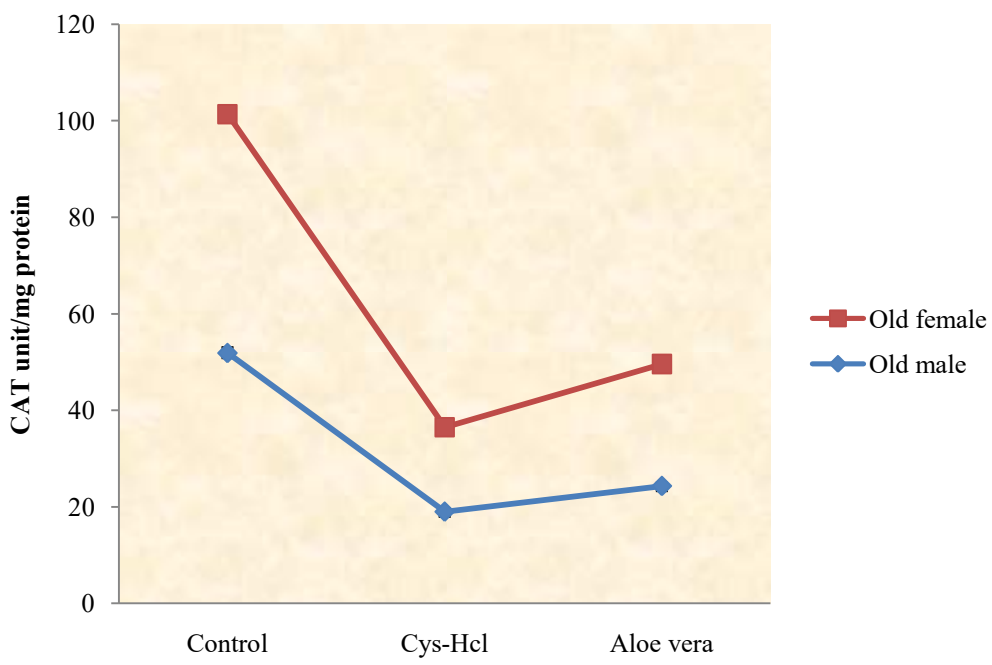
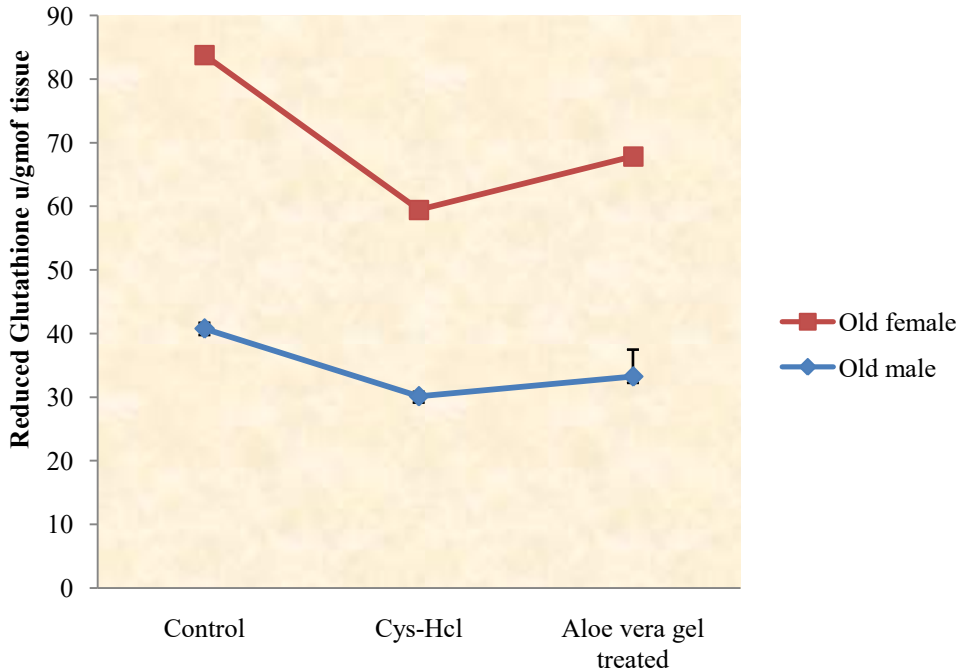
Graph No.1 Effect of Aloe vera gel on SOD activity (unit/mg protein /hr) of Cysteamine induced duodenal ulcer in old male and female mice



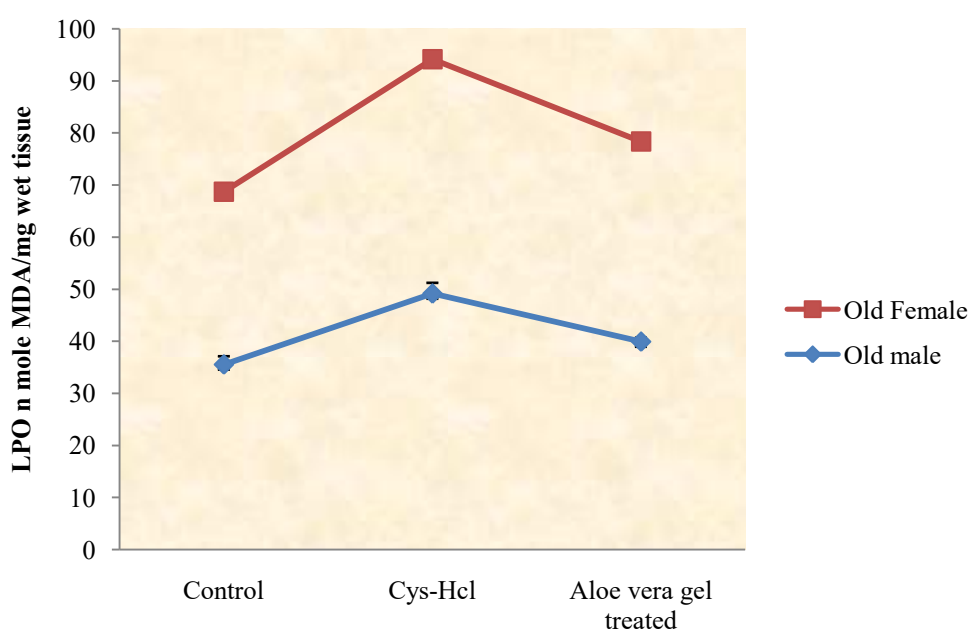
Graph No. 2 Effect of Aloe vera gel on CAT activity (unit/mg protein) of cysteamine induced duodenal ulcer in old male and female mice



Graph No.3 Effect of Aloe vera gel on reduced glutathione (u/gm of tiissue) of cysteamine induced duodenal ulcer in old male and female mice.



Graph No.4 Effect of Aloe vera gel on LPO content (n mole MDA /mg wet tissue) of cysteamine induced duodenal ulcer in old male and female mice



All 1, 2 3 and 4 graphs represented SOD, CAT, GSH and LPO content was found to be almost equal in

both male and female old mice. The increase in the SOD, CAT, GSH and decreased LPO activity in duodenum of *Aloe vera* treated both old male and female mice was highly significant as compared to cysteamine – HCl induced ulcerated mice both male and female mice.

The present study showed that cysteamine – HCl induces alterations in the antioxidants enzyme activities which were recovered at normal level by the treatment of *Aloe vera* gel. Defensive factors consist of several free radical scavenger enzymes such as decreased reduced glutathione, catalase (CAT), Super Oxide Dismutase (SOD) activity while increased LPO activity in ulcerated mice. The our result consistent with the abnormal oxidative metabolism generate high amount of free radicals occurs in the pathogenesis of ulcerative colitis (Tian, *et al.,*2017).The gastrointestinal tract is potentially vulnerable to oxidant injury due to a low concentration of antioxidant enzymes, which are mainly localized in epithelial cells (Grisham, *et al*.,1990) The our present study injection of Cys –HCl resulted in the formation of ulcer in both sexes of mice which was characterized by alterations in the activities of some enzymes reflecting the development of ulcer due to oxidative stress these findings consistent with the oxidative stress results into the generation of ROS and these free radicals significantly responsible for the development of ulcer (Tandon ,*et al*.,2004; Srinivas, *et al.,*2011)., such as gastritis, peptic ulcerations or multiple pathologies such as gastric adenocarcinoma (Chakraborty,*et al*.,2012; Uduak, *et al*., 2012). These ROS are responsible for the oxidation of tissues leading to lipid peroxidation and tissue damage. Oxidative damage is considered to be an important factor in the pathogenesis of ulcer as evidenced in different experimental and clinical models (Joshi, *et al*., 2007).Decreased SOD activity is an indicator of impairment of the protective mechanisms and significantly contributes to cell damage.SOD scavenges the super oxide radical O_2–, one of the reactive oxygen species (ROS) responsible for lipid per oxidation (Fridovich, 1986).The concentration of lipid peroxidation products malondialdehyde (MDA) increased as a results of the formation mucosal damage may be due to the increased reactive oxygen species (ROS) with altered antioxidant defense system. As increased oxidative stress the hydrogen peroxide (H_2O_2) accumulates into high amount, catalase activity becomes decreased results into increased lipid peroxidation (Rodriquez –cabezas, *et al*., 2004) in comparison to the control groups.

In the present study increased Super Oxide Dismutase (SOD), catalase (CAT), GSH(reduced glutathione) while increased LPO activity by the treatment of *Aloe vera* gel.It is observed that the functional phytoconstituents of *Aloe vera* involved in the cytoprotection via strengthen activities of defensive factors such as mucosal resistance. Gastrointestinal tract entirely covered with mucosa which acts as protective barrier (Hiruma-Lima, *et al*., 2012) against various irritants (Borra, *et al*., 2011).Our results similar with the findings that mucus has antioxidant properties helps to overcome mucosal damage caused by reactive oxygen species (Reptto and Llseuy, 2002). *Aloe vera* gel treatment enhance the protective antioxidant enzymes content by the removing the free radicals by its rich antioxidant phytoconstituents help to prevent the cell damages in the body (Steencamp and Stewart, 2007).The present study coincides with the previous (Punitharaj, *et al*., 2006) reports of oral administration of *Aloe vera* gel caused significant increase in enzymatic antioxidants such as GPX, catalase, SOD. All these effects were significantly reversed by treatment with *Aloe vera* gel that supporting a close relationship between free radical scavenging activity due its anti-oxidant properties and anti-ulcerogeinc activity.

IV. ACKNOWLEDGEMENT:

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ANGIOSTATIC EFFECT OF BARK EXTRACT OF *OCIMUM SANCTUM* ON ANGIOGENESIS BY USING CHICK CHORIOALLANTOIC MEMBRANE, IN OVO

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ABSTRACT: *Ocimum sanctum, the 'queen of herbs'- Tulsi, is used in ethanomedicines science the human civilization. The acetone bark extract of O. sanctum was tasted for its angiogenesis potency by using chick chorioallantoic membrane (CAM) in ovo. There was significant reduction in number of vitelline blood vessels. The histological and immunohistochemical study of treated CAM was compared with that of normal, sham and DNS control. The results indicate that there are reduction in capillary plexus and the pro-angiogenic factor, vascular endothelial cell factor (VEGF). Hence the plant having angiostatic activities which can be used in therapeutic angiogenesis to cure pathological conditions like cancer.*

Keywords: *Angiogenesis, Chick chorioallantoic membrane, O. sanctum*

I. Introduction

Angiogenesis is the process of neovasculature from pre-existing blood vessel. It begins in organogenesis during development. The neovasculature is of two types- sprouting and intussuptive. John Hunter first of all reported scientific insights in the field of angiogenesis in 1794, while Judah Folkman is the pioneer for describing the therapeutic angiogenesis (1971). Angiogenesis plays important role in physiology and pathology. It is essential for wound healing and cyclic changes in female reproductive system. Angiogenesis is almost stopped in adult and is controlled by switches or factors like pro-angiogenic and anti-angiogenic factors. These both factors are always kept in balance. The imbalance is reported in pathological conditions. Excessive angiogenesis is observed in cancer, while insufficient angiogenesis is in strokes and ulcer like complications.

Since the work by Folkman there is unending quest for discovery of angiogenic as well as angiostatic substances which can be used for therapeutic angiogenesis. There are various biomolecules used and tasted for its angiogenesis activity. 'Holy Basil', 'Tulsi'- *Ocimum sanctum* is used in various ethanomedicines to cure several pathological conditions. It is traditional medicinal plant used for treatment of various diseases. It is used as anti-dibetic, anti-fungal, anti-microbial, anti-cancerous, cardioprotective, immunoprotective, anti-helminthic, antiseptic, analgesic as well as tonic (Kirtikar and Basu, 1965).There are various angiogenesis assays used by the scientists but each one having its own limitations. Out of these several chick chorioallatoic membrane (CAM) is mostly used and the best angiogenesis assay (Richchrdson and Singh, 2003).

II. MATERIALS AND METHODS

The bark of *O. sanctum* was shed dried, powdered and extracted in acetone. The yied of the extract is 1.05^%. The concentrated stock solution is prepared in acetone. At the time of treatment the solution was prepared in medicated saline DNS (Mark- bioscience Ltd. Goa- G 2173003).

The chick CAM assay was used for screening the effect of acetone bark extract. The window method is used (Korn and Cramer, 2007) with some modifications. Fresh fertilized eggs of Gallus gallus was collected and grouped into- normal sham (operative control), DNS control and treated. The eggs were kept in sterilized incubator adjusted at 37- 39^{o}C with75% humidity. The sham is operative control, DNS control eggs were injected with 1 ml of DNS and treated eggs were injected with 0.5 mg/ml acetone bark extract in DNS at 48 hrs of incubation. The CAM was examined after 144hrs of incubation.

The pieces of CAM from normal, sham, DNS control and treated were fixed in 2% CAF. These are processed for histology and immunohistochemistry. The sections were stained by HE method for histology and for anti-VEGF for immunohistochemistry by DAB staining.

III. RESULTS AND DISCUSSION

In the present investigation the effect of acetone bark extract of *o. sanctum* was studied after 144

hrs of incubation, at the end of 6ED. The sprouting type of angiogenesis was evaluated morphometrically by counting the vitelline blood vessels, histologically and immunohistochemically. There was significant decrease in number of secondary and tertiary blood vessels in treated CAM as compare to normal, sham and DNS controlled CAMs (Shah and et al, 2015).

The CAM is histologically differentiated into outer ectoderm, inner endoderm and mesoderm is sandwiched between them. For respiratory exchange, in chick CAM capillary plexus are developed near the chorionic ectoderm and latter on interdigitate in chorionic ectoderm (Bruton and Palmer, 1989). Hence chick CAM capillary plexus provides excellent model to study angiogenesis.

The treated CAM shoes decreased number and size of capillary plexus as shown in the plate I. There was no significant change in the histology of sham as compare to normal and DNS control CAMs. The treated CAM is with less number of capillary plexus and decreased mesoderm. The ectoderm is separated from mesoderm at some places as shown in Plate I, D. The same findings were reported by Melkonian et al (2000). The capillary plexus were failed to mature and form blood vessel during angiogenesis. There are various phytoconstituents in *O. sanctum* including eugenol, ursolic acid, asgenin, luteolin, oleic acid (Norr and Wanger, 1992). These phytoconstituents acts as anticancerous, antiproliferative and having pro-apoptotic effect (Jha et al, 2012). This angiostatic effect of extract is due to decreased amount of angiogenic factors or increased amount of angiostatic factors. VEGF is the key player in the process of angiogenesis. Immunohistochemical staining was employed to localize VEGF antigen expression during angiogenesis in normal, sham control, DNS control and treated chick CAMs using DAB staining. It was reported that there was decreased staining intensity in treated CAM (Plate I, H). Hence the mechanism of action of phytoconstituents may in include inhibition of VEGF receptors. There are many chemical drugs used in medicines for therapeutic angiogenesis with its side effects. These phytoconstituents are having minimum side effects. The natural products like phytoconstituents contain cocktail of biological chemicals that can acts on multiple pathways that controls tumor angiogenesis (Sager et al, 2006).

IV. CONCLUSION

This in ovo study of acetone bark extract of *O. sanctum* on angiogenesis in chick CAM uncovers angiostatic activity of the plant extract. Further clinical trials and research is required for study and plan the dose of such natural source to treat malignancy.

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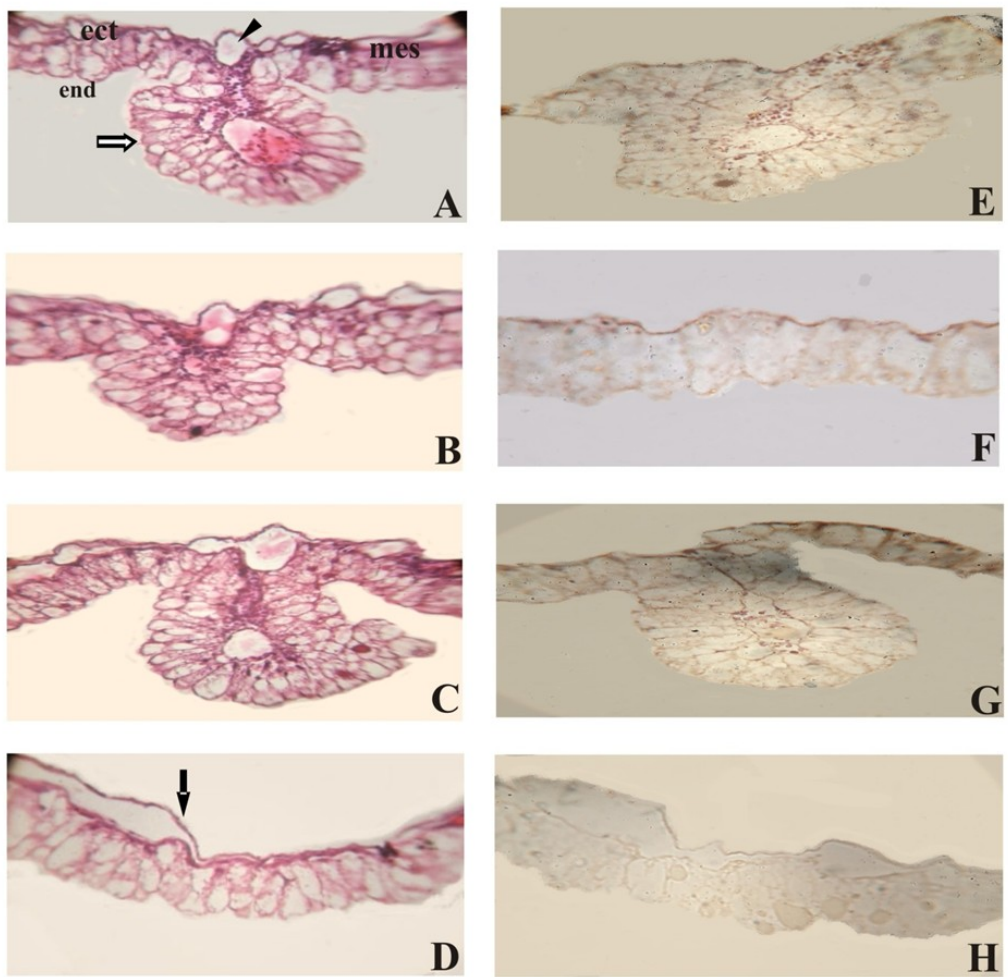
PLATE I



Effect of acetone bark extract of *O. sanctum* on chick CAM

A to D- T. S. of HE stained CAM (X450x)
E to H- T. S. of CAM immunohistochemisry for VEGF (X450x)
ect- Ectoderm, end- Endoderm mes- Mesoderm
Arrow- Blood vessel Arrow head- Capillary plexus
Solid arrow- Seprated ectoderm

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EFFECT OF DUAL INOCULATION OF AM FUNGI AND *AZOTOBACTER* ON YIELD OF BRINJAL *(SOLANUM MELONGENA* L.*)* VAR. KRISHNA.

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ABSTRACT: *The present investigation was undertaken with view to study the effect of dual inoculation of AM fungi and Azotobacter on yield of Brinjal (Solanum melongena L.) var. Krishna. The field experiment was conducted during Kharif 2016 in randomized block design with three replications. The carrier based Azotobacterculture was obtained from Department of Microbiology, Dahiwadi College Dahiwadi. The AM inoculation of Glomus fasciculatumwas obtained from University of Agricultural Sciences Dharwad (Karnataka). Observations were recorded only in terms of fruits yield quintals per hectare.*

Keywords: *Dual inoculation of AM fungi, Azotobacter and Brinjal.*

I. INTRODUCTION

India is a leading vegetable producing country in the world; and ranks second next to China. The country being blessed with the unique gift of nature's diverse climate and distinct seasons, which make it possible to grow an array of vegetables number exceeding more than hundred types.

Brinjal or Egg plant (*Solanum melongena L.)* var. Krishna is one of the most common, popular and principle vegetable crop grown in India and also other parts of the world. It can be grown in almost all the year round. It is highly productive and usually find its place as the "Poor man's crop". In India it is being consumed as a cooked vegetable in various ways. Maharashtra, Gujarat, Uttar Pradesh, Karnataka, Tamilnadu, Punjab, Bihar and Madhya Pradesh are major brinjal growing states in India.

Brinjal is staple vegetable in our diet since ancient times. It occupies significant place in both vegetarian and non-vegetarian diets. As a food, it is used in variety of ways depending on the economic conditions and the eating habits prevalent in different parts of India. The harvested fruits are used as a vegetable or in curries. It has also considerable ayurvedicmedicinal properties. Brinjal is good for diabetic patients & also excellent remedy for liver complaints[4].

There are some verities of Brinjal developed by IARI are Pusa Purple Long, Pusa Purple Round, Pusa Purple Cluster, Pusa Kranti and Pusa Amol. Whereas Vaishali, Manjari Gota, Pragati and Krishna are recommended by M.P.K.V. Rahuri (MS).

In the recent years, due to shortage of expensive nitrogenous fertilizers, due emphasis is given towards the use of microbial inoculants like *Azotobacter*which is known to produce growth promoting substances.

The research carried out during the last decades has established that, Vesicular Arbuscular Mycorrhiza (VAM) improve the plant growth through increased uptake of phosphorus[1]. The growth response is normally associated with increased supply of phosphorus from the soil[2].

Considering the importance of AM fungi and *Azotobacter*in saving nitrogen and phosphorus requirements of Brinjal crop the present investigation was carried out to study the effect of AM fungi in combination with, *Azotobacter* on yield.

II. MATERIALS AND METHODS

The study was conducted during Kharif 2016

Seeds**:-** Genetically pure seeds of Brinjal cv. Krishna were obtained from the vegetable breeder, All India-Co-ordinated Vegetable Improvement Project, M.P.K.V. Rahuri. (b) Inoculants**:-**The carrier based *Azotobacter* culture was obtained from the Department of Microbiology, Dahiwadi College Dahiwadi and used for field experiment. The Arbuscular Mycorrhizalinoculums of *Glomus fasciculatum* was obtained from University of Agricultural Sciences, Dharwad (Karnataka). (c)Fertilizers:-Recommended dose of fertilizers is 100kgN, 50Kg $P_2$05, and 50 kg of K_2O/hectare.

1) Field experiment

A field experiment was conducted to study the effect of dual inoculation of AM fungi and *Azotobacter*on growth, nutrient uptake and yield of brinjal.

2) Experimental Design

Randomized Block Design (RBD) was used for conducting the experiment.

3) Treatment Details

T_1- Recommended dose of N & P fertilizer
T_2- 75% Recommended dose of N & P fertilizer
T_3- 50% Recommended dose of N & P fertilizer
T_4-Recommended dose of N fertilizer + *Azotobacter*
T_5-75% Recommended dose of N fertilizer + *Azotobacter*
T_6- 50% Recommended dose of N fertilizer + *Azotobacter*
T_7 - Recommended dose of P fertilizer + AM
T_8 - 75% Recommended dose of P fertilizer + AM
T_9 - 50% Recommended dose of P fertilizer + AM
T_{10} - Recommended dose of N & P fertilizer + *Azotobacter* + AM
T_{11} - 75% Recommended dose of N & P fertilizer + *Azotobacter* andAM
T_{12} - 50% Recommended dose of N & P fertilizer + *Azotobacter* &AM
Note :- K applied as per recommended dose for all treatments.

Fig. 1 Plan of layout

RI

T_3	T_7	T_{12}	T_{10}	T_2	T_4
T_5	T_9	T_6	T_1	T_{11}	T_8

RII

T_{10}	T_2	T_1	T_8	T_6	T_{11}
T_4	T_5	T_7	T_3	T_{12}	T_9

RIII

T_2	T_4	T_8	T_5	T_{10}	T_6
T_9	T_{12}	T_{11}	T_7	T_3	T_1

Spacing : 90 x 75 cm and Variety : Krishna
Treatment : 12 Gross plot size : 3.6 x 3.75 cm
Replication : 3 Net plot size : 1.80 x 2.25 m.

4) Soil

The soil of the experimental plot was medium black, clay loam in texture. The topography of the experimental site was fairly uniform, leveled and well drained

5) Cropping history of the plot

During the previous season Sugarcane had taken in the plot.

6) Fertilizer application

The recommended dose of fertilizer was 100kg N, 50kg P_2O_2 and 50kg K_2O per hectare. The N applied through Urea in three graded levels i.e. 100% of R.D., 75% R.D. and 50% of R.D.. The P_2O_5 applied through Single super phosphate in three graded levels i.e. 100% of R.D., 75% of R.D. and 50% R.D.. The K_2O applied as per 100% R.D. through Murate of potash for all treatment.

7) Seeding inoculation and transplanting

Before transplanting the total seedlings were divided as per treatments. Seeding was treated with culture by slurry method[5]. Seeding roots were dipped in slurry for 15 minutes. The inoculated seedlings were transplanted In respective plot as per the plan of layout (fig.1.)

Culture of AM fungi inoculated by applying 2500 spores per plant directly, at the time of transplanting

8) Irrigation and interculturing operations

The experimental plot was irrigated after transplanting and subsequent irrigation was given at an interval of eight to ten days up to the harvesting. The weeding operation was carried out whenever necessary.

9) Plant protection measures

The plant protection measures were undertaken as per the recommendation. After transplanting, the sprays of Monocrotophos (36 WSC) and Dithane M-45 @ 0.25 per cent and Profenos 2 ml/litre were given at an interval of 15 days as precautionary measure against insects such as thrips, aphids and shoot

and fruit borer and diseases like bacterial blight and little leaf.

10) Fruit yield –

The picking of brinjal fruits was done regularly and yield of fruits recorded in quintals per hectare.

11) Statistical analysis

The data was subjected to statistical analysis by following the standard methods for analysis of variance, standard error for the treatment means and critical difference at 5 percent level of significance worked out for drawing precise conclusions[4].

Table 1. Effect of dual inoculation of AM fungi and Azotobacter on yield (q./ha) of brinjal crop :

Sr. No.	Treatments	Yields (q./ha)
1	T_1 - Recommended dose of N & P fertilizer	285.31
2	T_2 - 75% Recommended dose of N & P fertilizer	248.89
3	T_3 - 50% Recommended dose of N & P fertilizer	212.63
4	T_4 - Recommended dose of N fertilizer + *Azotobacter*	297.34
5	T_5 - 75% Recommended dose of N fertilizer + *Azotobacter*	262.22
6	T_6 -50% Recommended dose of N fertilizer + *Azotobacter*	234.07
7	T_7 -Recommended dose of P fertilizer + AM Fungi	287.22
8	T_8 - 75% Recommended dose of P fertilizer + AM Fungi	254.81
9	T_9 - 50% Recommended dose of P fertilizer + AM Fungi	228.15
10	T_{10} - Recommended dose of N & P fertilizer + *Azotobacter* + AM Fungi	311.51
11	T_{11} - 75% Recommended dose of N & P fertilizer + *Azotobacter* &AM Fungi	305.26
12	T_{12} - 50% Recommended dose of N & P fertilizer + *Azotobacter* &AM Fungi	244.40
	Mean	264.31
	S.E. +-	4.35
	C.D. at 5%	13.10

III. RESULTS AND DISCUSSION

The effect of dual inoculation of Am fungi and *Azotobacter*on yield (q/ha) was recorded at the harvest and presented in Table. 1

The data revealed that treatment T_{10}*i. e*. recommended dose of N and P fertilizer + *Azotobacter* and AM fungi recorded significantly higher yield of Brinjal (311.51 q./ha) as compared to other treatments, whereas it was on per with treatment T_{11} i.e. 75% Recommended dose of N & P fertilizer + *Azotobacter* + AM fungi (305.26 q./ha)

It was also observed that treatment T4recommended dose of N fertilizer + *Azotobacter* recorded significantly higher yield in Brinjal (297.34 q./ha) .It was on par with treatment t_7. recommended dose of P fertilizer + AM fungi (287.22 q./ha) recommended dose of N & P fertilizer (285.31 q./ha).

The results in general indicated that dual inoculation AM fungi and *Azotobacter* increased number of fruits per plant i.e. treatment T_{10} recommended dose of N & P fertilizer + *Azotobacter*& Am fungi (22.60) and yield i.e. T_{10}(311.51 q./ha) of brinjal crop over other treatments.

IV. CONCLUSION

The results of field experiment showed that dual inoculation of AM fungi and *Azotobacter* along with either 100% or 75% recommended dose of fertilizers significantly increased yield (q./ha) of brinjal crop.

The results therefore suggests the use of dual inoculation of AM fungi and *Azotobacter* along with either 100% or 75% recommended dose of fertilizers for better productivity of Brinjal crop. Mare or less similar results were obtained at dual inoculation of AM fungi and *Azotobacter* with either 100% or 75% recommended dose of fertilizer.

Investigation showed that dual inoculation of AM fungi and *Azotobacter* along with 75% recommended dose of fertilizer can be used for cultivation of Brinjal. It is also concluded that 25% recommended dose of N and P fertilizer can be saved by inoculating seeding of brinjal with AM fungi and *Azotobacter* as low cost technology.

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A NEW METHOD TO SOLVE BALANCED ASSIGNMENT PROBLEM

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ABSTRACT: *If number of rows and number of columns are same then an Assignment Problem (AP) is said to be Balanced Assignment Problem (BAP) otherwise, it is said to be UBAP. In this paper, we have proposed a method to solve an BAP. This method includes two parts. First is to obtain an Initial Basic Feasible Solution (IBFS) and second part is to test optimality of an IBFS. We have proposed a new method Row Column Penalty Assignment Method (RCPAM) to obtain an IBFS of a Balanced Assignment Problem. optimality of an IBFS is tested by using Non Besic Smallest Effectiveness Method (NBSEM). We can solve an assignment problem of maximization type using this method in opposite sense. Using this method, we achieve the goal with less number of computations and steps. Further we illustrate a method by suitable examples.*

Keywords: *AP, BAP, UBAP, IBFS, RCPAM, NBSEM*

1. Introduction:

The assignment problem is a special case of the transportation problem where the resources are being allocated to the activities on a one-to-one basis. Thus, each resource (e.g. an employee, machine or time slot) is to be assigned uniquely to a particular activity (e.g. a task, site or event). In assignment problems, supply in each row represents the availability of a resource such as a man, machine, vehicle, product, salesman, etc. and demand in each column represents different activities to be performed such as jobs, routes, factories, areas, etc. for each of which only one man or vehicle or product or salesman respectively is required. Entries in the square being costs, times or distances. The assignment method is a special linear programming technique for solving problems like choosing the right man for the right job when more than one choice is possible and when each man can perform all of the jobs. The ultimate objective is to assign a number of tasks to an equal number of facilities at minimum cost (or maximum profit) or some other specific goal.

Let there be 'm' resources and 'n' activities. Let c_{ij} be the effectiveness (in terms of cost, profit, time, etc.) of assigning resource 'i' to activity 'j' (i = 1, 2, …., m; j = 1, 2,…., n). Let $x_{ij} = 0$, if resource 'i' is not assigned to activity 'j' and $x_{ij} = 1$, if resource 'i' is assigned to activity 'j'. Then the objective is to determine x_{ij}'s that will optimize the total effectiveness (Z) satisfying all the resource constraints and activity constraints.

2. Mathematical Formulation:

Let number of rows = m and number of columns = n. If m = n then an AP is said to be BAP otherwise, it is said to be UBAP.

If m = n then mathematically the BAP can be stated as follows:

$$\text{Minimize, } Z = \sum_{i=1}^{m}\sum_{j=1}^{n} c_{ij}x_{ij} \qquad (2.1)$$

$$\text{Subject to, } \sum_{j=1}^{n} x_{ij} = 1,\ i = 1, 2, \ldots, m \text{ (availability constraints),} \qquad (2.2)$$

$$\sum_{i=1}^{m} x_{ij} = 1,\ j = 1, 2, \ldots, n \text{ (requirement constraints),} \qquad (2.3)$$

$$\text{and} \quad x_{ij} = 0 \text{ or } 1, \text{ for all i and j.} \qquad (2.4)$$

Presently, an AP can be solved by using one of the four methods, (i) Enumeration method, (ii) Simplex method (iii) Transportation method and (iv) Hungarian method**.** Among these four methods Hungarian method can be used as an efficient method for finding an optimal solution of an AP. But, this method also requires more number of computations and steps, Hadley (1997), Taha (2008), Kanti Swarup et al. (2008), Gupta and Hira (2010), Sharma (2010). To solve an AP of maximization type, it is require to convert it into minimization type. This leads to do more number of computations and steps to get an optimal solution.

Kore (2008) and Kore (2012) made an attempt to solve unbalanced transportation and an assignment problem without balancing it. As AP is a particular case of TP in this paper, we have proposed a new method to solve an AP, which overtakes the problem of degeneracy of transportation method. Using our new method we get, optimal solution of an AP, with less number of computations and steps. We can illustrate the comparison between our new method and Hungarian Method by solving various types of APs.

3. A) Algorithms of the new method to obtain an IBFS:

3.1 Row Column Penalty Assignment Method (RCPAM):

***Step 1*:** For each row and column determine a penalty by taking the difference between the smallest and next smallest effectiveness.

***Step 2*:** Let maximum of row penalties = A and maximum of column penalties = B.

a) If A ≠ B then observe the smallest effectiveness in the row and column corresponding to A and B respectively. Select largest of the smallest effectiveness encircle it, cross out the corresponding row and column. If there is a tie in largest of the smallest effectiveness and all of them are corresponding to either A or B then select that largest effectiveness corresponding to which next to next smallest effectiveness is maximum. Otherwise, select that largest effectiveness corresponding to which the sum of row penalty and column penalty is maximum. If there is again tie then select one of them randomly. If all A are zero or all B are zero then consider penalties B or A respectively.

b) If A = B then observe the smallest effectiveness in the row and column corresponding to A and B respectively. If smallest effectiveness corresponding to A and B are in the same row or column then select largest of the smallest effectiveness. Otherwise, select that smallest effectiveness corresponding to which sum of row penalty and column penalty is minimum. If there is a tie then select smallest of the smallest effectiveness. If there is again tie then select one of them randomly, encircle it, cross out corresponding row and column.

***Step 3* :** Repeat step 1 and step 2 until only one row and column is remain uncrossed, encircle that effectiveness which satisfies the last row and column simultaneously, cross out the last row and column.

Note:

i. If an AP is of maximization type then use RCPAM to obtain an IBFS in opposite sense.
ii. For using the NBSEM to test an optimality of IBFS of the AP select non-basic smallest effectiveness corresponding to which row and column have a basic cell.
iii. For an AP of maximization type, to test an optimality of an IBFS we can use the NBSEM in opposite sense.

C) Algorithm of the New Method to solve an AP

Step 1: Express the given AP in tabular form.
Step 2: Check whether the AP is BAP or UBAP.
Step 3: If an AP is BAP then obtain an IBFS using RCPAM.
Step 4: Optimize an IBFS of AP by using NBSEM to get an optimal solution of given AP.
Step 5: Write optimal solution and the optimum value of objective function (Z).

4. Applications of the New Method:

We illustrate the effectiveness of the new method by solving various types of APs.

Example 4.1: Consider the following AP of minimization type, Gupta PK and Hira DS (2010):

	1	2	3	4	5
1	15	10	25	25	10
2	1	8	10	20	2
3	8	9	17	20	10
4	14	10	25	27	15
5	10	8	25	27	12

Here, m = 5 and n = 5 i.e. m = n, the problem is BAP.

Using the RCPAM to obtain an IBFS and testing its optimality by using the NBSEM we get,

Table 4.1 IBFS

	1	2	3	4	5	R.P.	
1	15	10	25	25	(10)		(0) (5)
2	1	8	(10)	20	2		(1) (1) (9) (10)
3	8	9	17	(10)	10		(1) (2) (9) (3)
4	14	(10)	25	27	15		(4)
5	(10)	8	25	27	12		(2) (2) (15)
C. P.	(7) (7) (7)	(0)	(7) (7) (7) (7)	(0) (0) (0) (0)	(8) (8)		

Here, improvement in the present solution is not possible by introducing the non-basic smallest effectiveness 1 in the basis.

The present solution is an optimal solution to the given BAP.

The optimal solution is assign, 1→ 5, 2→ 3, 3→ 4, 4→ 2, and 5→1.

The optimal value of Z is, Z_{min} = 10 + 10 + 20 + 10 + 10 = 60.

By solving the above BAP by using Hungarian method after 4 steps we get,

Table 4.2 Optimal Solution

	1	2	3	4	5
1	5	2	6	3	(0)
2	×0	9	(0)	7	1
3	×0	3	×0	(0)	2
4	2	(0)	4	3	3
5	(0)	×0	6	5	3

Hence, the optimal solution is assign, 1 → 5, 2 → 3, 3 → 4, 4 → 2 and 5 → 1.

The optimum value of Z is, Z_{min} = 10+10+20+10+10 = 60.

Note:

i. For above example we get an optimal solution by using our new method in 1 step.

ii. By using Hungarian method we get an optimal solution in 4 steps.

iii. Since steps are more and computations are more, the time required to solve the above BAP by using Hungarian method is more than the time required by using our new method.

5. Findings:

i. If an IBFS of the BAP is obtained by using RCPAM and it is optimized by using NBSEM method then the least possible optimum value of Z is achieved.

ii. Using our new method to solve the BAP we get, optimal solution quickly, without changing the order of assignment table, with less number of steps, iterations and computations.

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FUZZY EXPLORING SIMPLE ADDITIVE WEIGHTING METHOD FOR MAINTENANCE STRATEGY SELECTION PROBLEM: CASE STUDY

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ABSTRACT: *This paper presents and proposes a different approach of selection an efficient maintenance strategy of material handling equipments in Shiv Ganga cement pipe industy, Bhose, Tal- Miraj (India) using Fuzzy Simple Additive Weighting (FSAW) method. In this paper by the experts weights are assigned in linguistic variables, these linguistic variables are translated into triangular fuzzy numbers (TFN) to the Multi-Criteria Decision-Making (MCDM) problem. Basic six types of maintenance strategy are corrective maintenance, preventive maintenance, condition based maintenance, opportunistic maintenance, predictive maintenance, and breakdown Maintenance and ten maintenance decision criteria namely quality, spare parts inventories, purchasing cost of spare parts, maintenance labour cost, Reliability, safety, Maintenance time, Facilities, cost of supporting equipment, and environment. In this paper breakdown maintenance strategy best one out of all maintenance strategy for material handling equipment.*

Keywords: *Multi-criteria decision-making, Maintenance strategy selection, Fuzzy SAW method, Linguistic variables, Triangular fuzzy Number.*

1. Introduction:

Proper maintenance of the plant equipment can significantly reduce the overall operating cost, while maximizing the productivity of the plant. The development of new technologies and managerial practices means that maintenance staff must be endowed with improving technical and managerial skills [1]. In many industries there is a strong incentive to maximize their plant and machinery lifetime. This means plant and machinery may be kept running beyond their original design lifetime to do so. Therefore, risk and reliability analysis has recently become a critical decision tool to optimize maintenance strategy in order to ensure safety and minimize costs [4]. Many companies think of maintenance as an inevitable source of cost. The maintenance strategy selection problem which is a multi-criteria decision-making (MCDM) problem faces the problem in estimating the related factors. To solve this problem, some approaches using fuzzy concepts have been proposed. In this paper, a new approach to the maintenance strategy selection problem is proposed which can determine the best maintenance strategy by considering the uncertainty level and also all the variety in maintenance criteria and their importance [2]. The Fuzzy Simple Additive Weighting (FSAW) for the evaluation of maintenance strategies is used, Triangular Fuzzy Number (TFN) in Fuzzy Simple Additive Weighting (FSAW) to model the uncertainty in the selection process is used and a fuzzy linguistic approach for the maintenance strategy selection problem is used.

2. Fuzzy Multiple Criteria Decision Making (FMCDM):

2.1 Review of FMCDM

The Fuzzy Multiple-criteria decision making (FMCDM), comprises a finite set of alternatives, amongst which the decision makers have to select, evaluate or rank according to the weights of a finite set of criteria (alternatives). Bellman and Zadeh [3] extend the decision making issues in to fuzzy environments, numerous works coped with uncertain and vague problem by utilizing fuzzy set theory. According to these literatures, FMCDM was mostly adopted in selection, evaluation and ranking, rarely used in solutions of prediction or forecasting. In this chapter we consider Simple Additive Weighting (SAW) method.

The characteristic and membership function of the triangular fuzzy number $\mu_{\tilde{A}(\chi)} = (L, M, U)$ are expressed by equation (1) and figure-2.1.

$$\mu_{\tilde{A}(\chi)} = \begin{cases} (X-L)/(M-L) & L \le X \le M \\ (U-X)/(U-M) & M \le X \le U \\ 0 & \text{Other wise} \end{cases} \quad (1)$$

$\mu_{\tilde{A}(\chi)}$

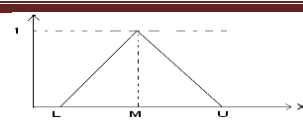

Fig.1- The membership function of the triangular fuzzy number

2.2 Linguistic variables

Zadeh stated that traditional quantification methods had difficulty reasonably expressing the condition that were overly complicated or hard to define, and thus linguistic sentiment offered a practical means of describing such situations. The theory of linguistic variable is given to express impression of spatial information and human cognation over the evaluation criteria. Seven linguistic variables for possible rating $\tilde{R}$ and weights $\tilde{W}$.

3. Proposed Methodology:

Using SAW method we assessed and identified set of maintenance approaches. The objective is to give rank of the maintenance approaches by evaluating their ability to provide information about the changes in the behavior of failure causes, which are used as criteria, as shown in table -3.1.

Table-3.1: Fuzzy decision matrix

Maintenance Approach (alternatives) ↓	Failure Causes (Criteria) C_1	C_2	...	C_j	...	C_n
	$\tilde{W}_1$	$\tilde{W}_1$	...	$\tilde{W}_j$	...	$\tilde{W}_n$
A_1	$\tilde{R}_{11}$	$\tilde{R}_{12}$	...	$\tilde{R}_{1j}$	...	$\tilde{R}_{1n}$
A_2	$\tilde{R}_{21}$	$\tilde{R}_{22}$	...	$\tilde{R}_{2j}$	...	$\tilde{R}_{2n}$
...	...	...	...	...	...	...
A_i	$\tilde{R}_{i1}$	$\tilde{R}_{i2}$	...	$\tilde{R}_{ij}$	...	$\tilde{R}_{in}$
...	...	...	...	...	...	...
A_m	$\tilde{R}_{m1}$	$\tilde{R}_{m2}$	...	$\tilde{R}_{mj}$	...	$\tilde{R}_{mn}$

The decision problem is composed of a matrix of $'m'$ maintenance approaches (alternatives) rated on a set of $'n'$ failure causes (Criteria) that are applicable to the case in question. $\tilde{W} = \left\{\tilde{W}_j ; \text{for } j = 1, 2 - - - n\right\}$ is a set of fuzzy numbers (weights) on the unit interval $[0,1]$ denoting the importance of considering the j^{th} failure (criteria) C_j.

$\tilde{R} = \left\{\tilde{R}_{ij}; \text{for } i = 1, 2, - - -, m; j = 1, 2, - - - n\right\}$ be a set of fuzzy number, also on the unit interval $[0,1]$ denoting the rating (capability) of the i^{th} maintenance approach on detecting changes in the j^{th} criterion using suitable measure.

4. Simple Additive Weighting (SAW) method:

It is the simplest and still the widest used in multiple criteria decision making (MCDM) method [38]. Here, each alternative is given a weight and the sum of all the weights must be one. Each alternative is assessed with regard to every criterion. The overall or composite performance score of an alternative is given by equation (2):

$$P_i = \sum_{j=1}^{n} W_j R_{ij} \qquad (2)$$

Previously, it was argued that SAW should be used only when the decision criteria's can be expressed in identical units of measure (e.g. only dollars, only pounds, only seconds etc.). However, if all the elements of the decision table are normalized, then SAW can be used for any type and number of attributes, in that case, equation (3) will take the following form:

$$P_i = \sum_{j=1}^{n} W_j \left(R_{ij}\right)_{Normal} \quad (3)$$

Where $\left(R_{ij}\right)_{Normal}$ represents the normalized value of R_{ij}, and P_i is the overall or composite score of the alternative A_i. The alternative with the highest value P_i is considered as the best alternative.

The attribute criteria can be beneficial or non-beneficial, when objective values of the attribute are available, normalized values are calculated by $\left(R_{ij}\right)_K / \left(R_{ij}\right)_L$ where $\left(R_{ij}\right)_K$ is the measure of the criteria for the K^{th} alternative, and $\left(R_{ij}\right)_L$ is the measure of attribute for the L^{th} alternative that has the highest measure of criteria out of all alternatives considered. This ratio is valid for beneficial criteria (e.g. profit) mean its highest measure is more describable for the given decision-making problem. By contrast, non-beneficial criteria (e.g. cost) is that for the lower measure are described, and the normalized values are calculated by $\left(R_{ij}\right)_L / \left(R_{ij}\right)_K$

If the restriction that the sum of all weights is to be equal to 1 is relaxed, then equation can be used and this method is called Simple Multiple Criteria Rating Technique (SMCRT).

$$P_i = \left[\sum_{j=1}^{n} W_j \left(R_{ij}\right)_{Normal}\right] / \sum_{j=1}^{n} W_j \quad (4)$$

In simple additive weighted method to assess weights for each attribute to reflect its relative importance to the decision. For a start, the criteria's are ranked in order of importance and 10 points are assigned to least important attribute. Then, the next least important criteria is chosen, more points are assigned to it and so on, to reflect their relative importance. The final weights are obtained by normalizing the sum of the points to one.

5. Numerical Example:

In a Shiv Ganga cement pipe industy, Bhose, Tal- Miraj (India), we can identify the failure causes (criteria) of machines components, shown in table 5.1.

The weight $\tilde{W}$ ="WEIGHT" estimated using the following possible ratings :{ very low (VL), low (L), more or less low (MLL), medium (M), more or less high (MLH), high (H), very high (VH)}. If the criteria is not applicable to the case at hand, we allocate a crisp value of zero denoted as none.

The output of the fuzzy inference system (FIS) is an assessment of the fuzzy variable $\tilde{R}$ ="RATE". This could be one of the following ratings :{ very weak (VW), weak (W), more or less weak (MLW), fair (F), more or less strong (MLS), strong(S), very strong (VS)}, and crisp value of zero denoted by none.

Table -5.1- Linguistic assessment of the weight $\tilde{W}$ for failure cases.

Failure causes (Criteria)		**Weight ($\tilde{W}$)**
C1	Purchasing Cost of Spare Parts	VH
C2	Maintenance Labour Cost	VH
C3	Maintenance Time	VH
C4	Reliability	VH
C5	Cost of Supporting Equipment	VH
C6	Environment.	L

Table-5.2 Linguistic assessment of $\tilde{R}$ and $\tilde{W}$

Failure causes (Criteria)	**Maintenance approaches (Alternatives)**						**Weight ($\tilde{W}$)**
	CM	**PBM**	**CBM**	**OM**	**PM**	**BM**	
C1	None	MLW	MLS	MLS	F	VS	VH
C2	None	MLW	MLS	MLS	F	VS	VH
C3	None	MLW	MLS	MLS	F	VS	VH
C4	None	W	F	MLS	W	VS	VH
C5	None	W	S	W	W	VS	VH
C6	None	W	W	W	W	VS	L

Using triangular fuzzy numbers (TFN) the linguistic fuzzy variables represented by $\tilde{W}$ = "WEIGHT" and $\tilde{R}$ = "RATE"

Table-5.3 linguistic weights to the alternatives are Table -5.4 linguistic weights to the criteria are

None	(0, 0, 0)	VH	(0.8, 0.9, 1.0)
VW	(0.1, 0.2, 0.3)	H	(0.7, 0.8, 0.9)
W	(0, 0.2. 0.3)	MLH	(0.6, 0.7, 0.8)
MLW	(0.1, 0.2. 0.3)	M	(0.4, 0.5, 0.6)
F	(0.4, 0.5, 0.6)	MLL	(0.2, 0.3, 0.4)
MLS	(0.5, 0.6, 0.7)	L	(0, 0.2, 0.3)
S	(0.7, 0.8, 0.9)	VL	(0.1, 0.2, 0.3)
VS	(0.8, 0.9, 1.0)		

The normalized decision matrix and normalized weighted decision matrix of SAW method is represented in table-5.5 and table-5.6.

Tabl-5.5 Normalized decision matrix

Criteria Alternative

	CM	PBM	CBM	OM	PM	BM	$\tilde{W}$
C_1	(0,0,0,)	(.2,.3,.4,)	(.5,.6,.7,)	(.5,.6,.7,)	(.4,.5,.6,)	(.8,.9,1,)	(.8,.9,1)
C_2	(0,0,0,)	(.2,.3,.4,)	(.5,.6,.7,)	(.5,.6,.7,)	(.4,.5,.6,)	(.8,.9,1,)	(.8,.9,1)
C_3	(0,0,0,)	(.2,.3,.4,)	(.5,.6,.7,)	(.5,.6,.7,)	(.4,.5,.6,)	(.8,.9,1,)	(.8,.9,1)
C_4	(0,0,0,)	(.1,.2,.3,)	(.4,.5,.6,)	(.5,.6,.7,)	(.1,2,.3,)	(.8,.9,1,)	(.8,.9,1)
C_5	(0,0,0,)	(.1,.2,.3,)	(.7,.8,.9,)	(.1,.2,.3,)	(.1,.2,.3,)	(.8,.9,1,)	(.8,.9,1)
C_6	(0,0,0,)	(.1,.2,.3,)	(.1,.2,.3,)	(.1,.2,.3,)	(.1,.2,.3,)	(.1,.2,.3,)	(.8,.9,1)

D =

Table-5.6 Normalized weighted decision matrix

Criteria Alternative

	CM	PBM	CBM	CM	PM	BM
C_1	(0,0,0,)	(.16,.27,.4,)	(.4,.54,.7,)	(.4,.54,.7,)	(.32,.45,.6,)	(.64,.81,1,)
C_2	(0,0,0,)	(.16,.27,.4,)	(.4,.54,.7,)	(.4,.54,.7,)	(.32,.45,.6,)	(.64,.81,1,)
C_3	(0,0,0,)	(.16,.27,.4,)	(.4,.54,.7,)	(.4,.54,.7,)	(.32,.45,.6,)	(.64,.81,1,)
C_4	(0,0,0,)	(.08,.18,.3,)	(.32,.45,.6,)	(.4,.54,.7,)	(.08,18,.3,)	(.64,.81,1,)
C_5	(0,0,0,)	(.08,.18,.3,)	(.56,.72,.9,)	(.08,.18,.3,)	(.08,.18,.3,)	(.64,.81,1,)
C_6	(0,0,0,)	(.08,.18,.3,)	(.01,.04,.09,)	(.8,.18,.3,)	(.08,.18,.3,)	(.08,.18,.3,)

D =

The utility value U_1 of SAW method is

$$U_1 = \begin{matrix} \text{CM} & \text{PBM} & \text{CBM} & \text{CM} & \text{PM} & \text{BM} \\ [(0,0,0,) & (.64,1.19,1.86) & (2.09,2.83,3.69) & (1.76,2.52,3.4) & (1.2,1.8\,9,2.7) & (3.28,4.23,5.3)] \end{matrix}$$

6. Concluding Remarks:

In this Paper, we proposed a framework based on fuzzy SAW approach for predicting most efficient maintenance strategy problem.

The linguistic variable is used to carry out the rating of the strategies with respect to the decided criteria. Fuzzy Simple Additive Weighted method has been established to select most efficient maintenance strategy under fuzzy decision criteria for given machine. This method is used for minimizing the number of failure and planned replacement.

By using the proposed fuzzy evaluation methodology, we are able to identify and select, in advance, the optimal maintenance strategy approach. Consequently we get higher product quality, improve efficiency and higher productivity and hence, better economy and profitability.

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ROOT OF MEAN OF SQUARES METHOD TO OBTAIN INITIAL BASIC FEASIBLE SOLUTION OF TRANSPORTATION PROBLEM

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ABSTRACT: *In this paper, we have proposed a new method Root of Mean of Squares (RMS) to find Initial Basic Feasible Solution (IBFS) of Transportation Problem (TP). After finding the IBFS we use Modified Distribution (MODI) method to optimize the IBFS. Using RMS we find that IBFS of most of the TP problems closer to optimal solution than using the other existing methods. We illustrate the same by suitable examples.*

Keywords: *TP, IBFS, MODI, RMS.*

1. Introduction:

The Transportation Problem is one of the most important applications of linear programming problem. These kind of problems helps to minimize the total cost of transporting a product from supply point to demand point. To obtain initial basic feasible solution North West Corner Method (NWCM), Least Cost Method (LCM) & Vogel's Approximation Method (VAM) are generally used and for optimality check we can use MODI method

In last few years many methods are proposed to find IBFS of TP, Kore B. G. (2008) proposed Row Penalty Method and Column Penalty Method for finding IBFS of TP, Das U. K. Khan A. R. Md. Ashraful Babu and Dr. Md. Sharif Uddin (2014) proposed Advanced VAM here finding the error of VAM. Palanivel M. and Suganya M. (2018) gave a new method to solve TP by using a new statistical method Harmonic Mean. Duraphe S, Modi G and Raigar S (2017) are proposed a new method for the optimum solution of a TP, here they calculated penalties by Using Arithmetic Mean. Sharma N. M. and Bhadane A.P. (2016) proposed an alternative method to NWCM for solving TP by using measures of dispersion the Coefficient of Range. Opara Jude, Oruh Ben Ifeanyichukwu, Iheagwara Andrew Ihuoma, Esemokumo Perewarebo Akpos proposed a new and efficient approach to find IBFS of a TP, here use the Coefficient of Variation.

In this paper RMS mean is used to find the IBFS of TP. The RMS is defined as the square root of the arithmetic mean of the squares of values. That is, the A.M of the squares of a set of number. The RMS is also known as the quadratic mean. The RMS used in both statistics and mathematics. Using RMS we find the IBFS of most of the TP closer to optimal solution than using the other existing methods. By this new approach we achieve the goal with less number of computations and easy to calculate. We illustrate the numerical examples for the new method & comparing these results to NWCM, LCM and VAM.

2. Mathematical Formulation:

The transportation problem is expressed as a linear transportation model as follows,

Minimize,

$$Z = \sum_{i=1}^{n}\sum_{j=1}^{m} C_{ij}X_{ij}$$

Subject to,

$$\sum_{j=1}^{m} X_{ij} = a_i , \qquad i = 1,2, \dots \dots \dots n \text{ (Supply)}$$

$$\sum_{i=1}^{n} X_{ij} = bj, \qquad j = 1,2, \dots \dots \dots \dots , m \text{ (demand)}$$

And $X_{ij} \geq 0$, for all i and j.

Where,

X_{ij} =The number of units shipped from i^{th} origin to j^{th} destination.

C_{ij} = per unit cost in shipping from i^{th} origin to j^{th} destination.

a_i = The amount available at i^{th} origin.
b_j =The demand available at j^{th} destination.

Formula of RMS:

$$C_{rms} = \sqrt{\frac{c_1^2 + c_2^2 + c_3^2 + \ldots\ldots\ldots c_n^2}{n}}$$

Where, C_{rms} = RMS Value (of costs whose mean is to be calculated)
$c_1, c_2 \ldots c_n$ = set of values
N = number of values.

3. Algorithm:

Step 1: Examine whether the transportation problem is balanced or not. If it is balanced then go to next step. If not then make it balanced by adding dummy row or column whatever is necessary.

Step 2: Find the RMS (Root of mean of square) value for each row as well as column and identify the one with maximum value.

Step 3: Compare the minimum of supply or demand whichever is minimum then allocate the min (supply or demand) at the place of minimum value of cost in related row or column.

Step 4: Repeat step 2 and 3 unless and until all the demands are satisfied and all the supplies are exhausted.

Step 5: Now total minimum cost is calculated as sum of the product of cost and corresponding allocated value of the supply or demand.

4. Examples:

4.1 Solve Following TP:

	D_1	D_2	D_3	Supply
S_1	2	7	4	5
S_2	3	3	1	8
S_3	5	4	7	7
S_4	1	6	2	14
Demand	7	9	18	34

Solution:

	D_1	D_2	D_3	Supply					
S_1	(5) 2	7	4	5,0	4.79	4.79	3.16	-	-
S_2	3	(2) 3	(6) 1	8,6,0	2.51	2.51	2.23	2.23	-
S_3	5	(7) 4	7	7,0	5.47	-	-	-	-
S_4	(2) 1	6	(12) 2	14,2,0	3.69	3.69	1.58	1.58	1.58
Demand	7,2,0	9,2,0	18,12,0	0					
	3.12	5.24	4.18						
	2.16	5.59	2.64						
	2.16	-	2.64						
	2.23	-	1.58						
	1	-	2						

The transportation cost is, **Z** = 5×2 + 2×3 + 6×1 + 7×4 + 2×1 + 12×2 = **76.**

4.2 Solve Following TP:

	D_1	D_2	D_3	D_4	Supply
S_1	2	4	5	8	52
S_2	5	7	6	7	59
S_3	16	20	10	12	28

S_4	19	18	17	28	94
Demand	40	55	68	70	233

Solution:

	D_1	D_2	D_3	D_4	Supply						
S_1	(23) 2	(29) 4	5	8	52,23,0	5.22	5.29	5.29	5.29	3.16	3.16
S_2	(17) 5	7	6	(42) 7	59,17,0	6.3	6.4	6.4	60.4	6.08	-
S_3	16	20	10	(28) 12	28,0	15	16.32	16.32	-	-	-
S_4	19	(26) 18	(68) 17	28	94,26,0	20.96	22.12	-	-	-	-
Demand	40,23,0	55,29,0	68, 0	70,42,0	40,23,0						
	12.7	14.04	10.6	16.13	12.7						
	12.7	14.04	-	16.13	12.7						
	9.74	12.44	-	9.25	9.74						
	3.8	5.7	-	7.51	3.8						
	3.8	5.7	-	-	3.8						

The transportation cost is Z = 23×2 + 29×4 + 17×5 + 42×7 + 28×12 + 26×18 + 68×17 = **2501**

Comparison between results of Existing method and proposed method.

Method	Example 1	Example 2
Proposed-RMS	76	2501
NWCM	102	3173
LCM	83	3002
VAM	80	2761
Optimal Solution	76	2484

5. Conclusions:

From comparison table, it is observed that the solutions obtained by the proposed method are less than that of existing methods wiz NWCM, LCM and VAM and very close to optimal solution as by MODI method. Also we can get the solution in lesser steps & calculations. Finally we can conclude that, by proposed method we can get better results as optimal or close to optimal solution directly in lesser steps.

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CUSTOMER SATISFACTION TOWARDS HERO MOTOCORP BIKES: A CASE STUDY IN VITA CITY

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ABSTRACT: *Any organization has to listen to its external customers and stakeholders. A number of studies have shown that the long-term success of a corporation is closely related to its ability to create and maintain loyal and satisfied customers, adapt to customer needs and changing preferences. In order to monitor customer satisfaction and to take an action for improving it. Sample of 200 customers of hero bikes user were surveyed with a structured questionnaire. In this paper we found that 28% of the customers to know the advertisement of hero bike through media.40% customers come under age 25-35 years i.e. we observed that the young and adult people used bikes.40%customers are business man which are prefer a hero model.49%customers opinion about hero bike is good.80% customers have not faced travelling problem about hero bikes. According to chi- square test for goodness of fit we find that there is significance difference between the factors like mileage, pick up, price, and design.*

Keywords: *Customer satisfactions, Hero MotoCorp bikes, Chi-square test.*

1. Introduction:

India has the largest number of two-wheelers in the world with 41.6 million vehicles. India has mix of 30 percent automobiles and 70 percent two wheelers in the country. India was the second largest two wheeler manufacturer in the world. Hero MotoCorp Limited is the world's largest manufacturer of two-wheeler. The company has four manufacturing facilities namely Dharuhera and Gurgaon in Haryana, Haridwar in uttarakhand and Neemrana in Rajasthan. The company is based in New Delhi, India.

The license raj that existed between the 1940's to 1980's in India did not allow were tightly controlled. This regulatory maze, before the economic liberalization, made business easier for focal players to have a seller market customer in India were forced to wait 12 years to buy a scooter from Bajaj. The CEO of Bajaj commented that he did not need a marketing department only a dispatch department. By the year 1990, Bajaj had a waiting list that was twenty –six times its annual output for scooters.

In the mid- 1980's the Indian government regulation changed and permitted foreign companies to enter the Indian market through minority joint ventures. The two wheelers market changed with four indo Japanese joint ventures. Hero Honda, TVS Suzuki, Bajaj Kawasaki And kinetic Honda. The entry of these foreign companies changed the Indian market dynamites form the supply side to the demand side with a larger selection of two- wheelers on the Indian market, consumers started to gain influence over the products they bought and raised higher customer expectations. The industry produced more models, styling options, prices and different fuel efficiencies. The foreign companies new technologies helped make the products more reliable and with better quality, Indian companies had to change to keep with their global counterparts.

2. Objectives:

i. To study the level of customer satisfaction with reference to Hero motocorp bikes in study area
ii. To study customer opinion about hero motocorp bikes in vita city.

3. Material and methods:

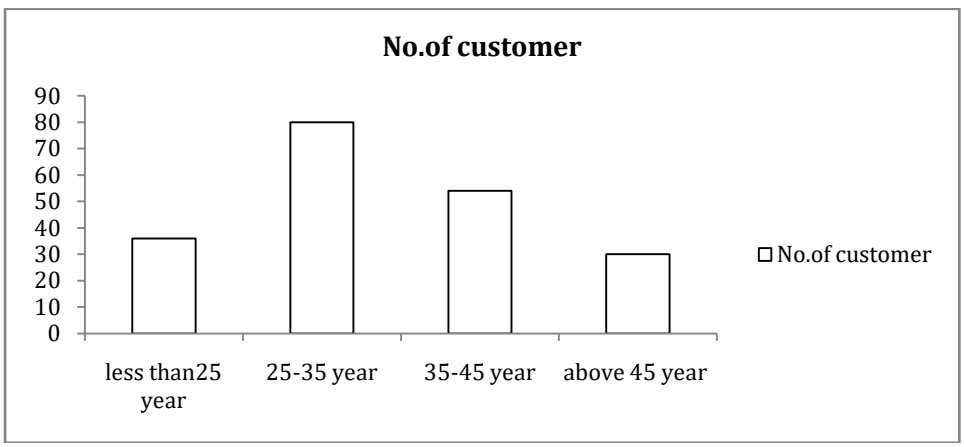
For the personal study the sample size is restricted to 200 respondents. These 200 respondents are users of hero bikes. The sample of 200 customers is selected by using simple random sampling method. Randomly 200 customers who use hero bike were selected for study purpose. The data collection was made on the basis of questionnaire method.

The statistical techniques chi-square test for goodness of fit is used to check level of customer satisfaction. The MS-EXCEL software is used for data analysis.

4. Data Analysis and interpretation:

Age- wise Classification of customers:

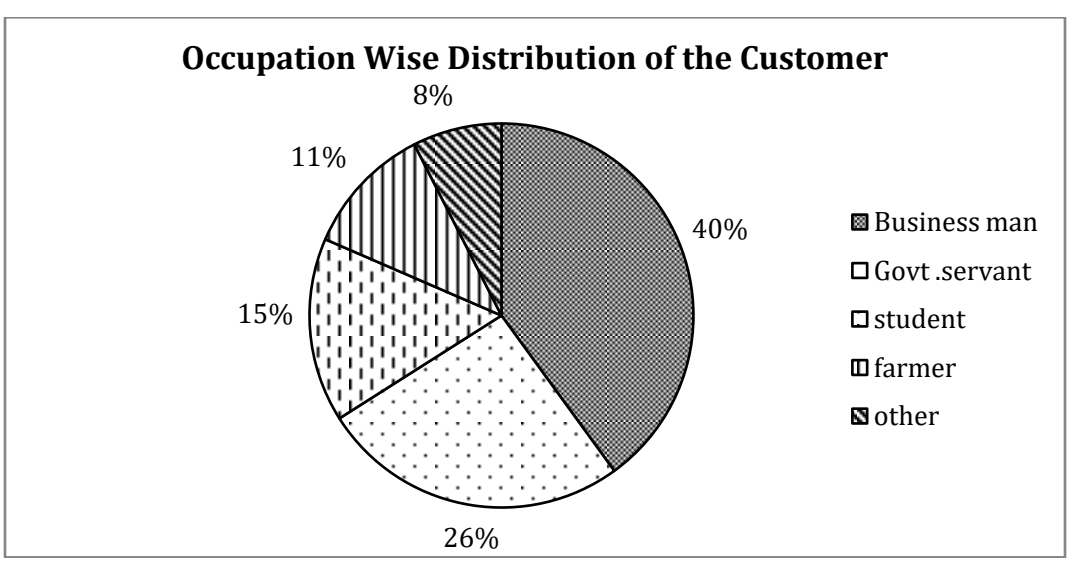
Age group	No.of customer	percentage
less than25 year	36	18 %
25-35 year	80	40 %
35-45 year	54	27 %
above 45 year	30	15 %



Interpretation: The above figure indicates that 18% customer of them come under age of less than 25 years,15% customer as under the age of above 45 years.27 % customers having age group of 35-45 and 40% customers are 25-35 years. Therefore, it can be observed that the young and adult people used bikes.

Occupation wise distribution of the customer:

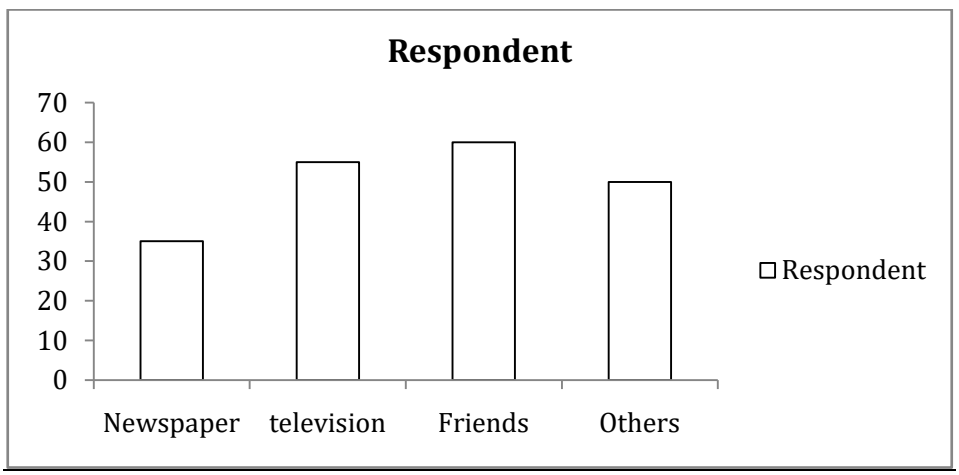
Occupation	No. of customer	Percentage
Business man	80	40 %
Govt .servant	52	26 %
student	31	15.5 %
farmer	22	11 %
other	15	7.5 %



Interpretation: The above table shows that, 11% are farmer, and 15.5% are having student.26% customers are govt servant. 40% customers are businessman and 7.5% others. It can be observed that, the business man and govt. servant prefer hero model.

Customer's hero bikes purchasing information source:

Source	Respondent	Percentage
Newspaper	35	17.5 %
television	55	27.5 %
Friends	60	30 %
Others	50	25 %



Interpretation: The above figure represents that 30%band 28% customers taking purchasing decision of hero bikes through friends and television.

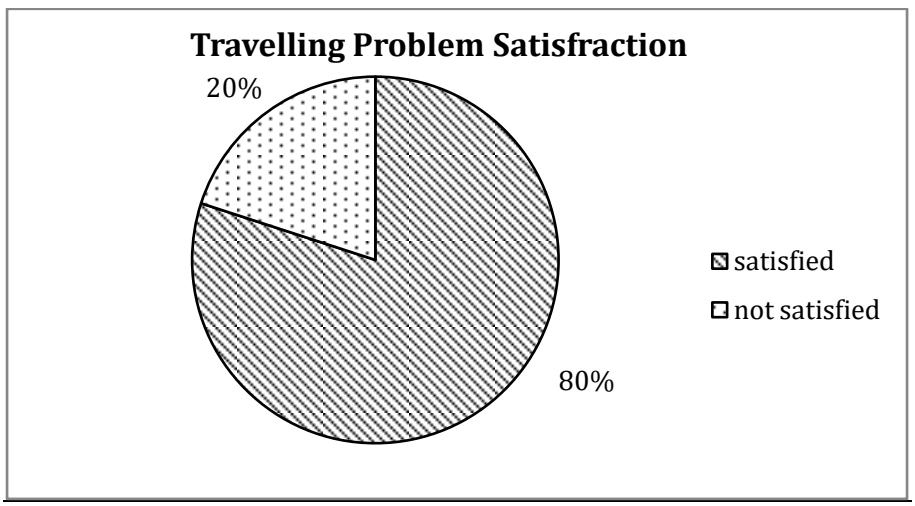
Customer opinion about Hero bikes:

Opinion	No. of Customer	Percentage
Good	98	49 %
Excellent	58	29 %
Average	36	18 %
Poor	8	4 %

Interpretation: The above table shows that maximum 49% customers opinion about the hero is good, 29%customers opinion is excellent. The minimum customers 18%have average opinion about the other 4%customers opinion is poor they have not satisfied.

Customer's satisfied about travelling problem in hero bikes

Opinion	No. of customers	Percentage
Satisfied	160	80 %
Not Satisfied	40	20 %



Interpretation: The above table represent customers opinion about travelling problem that 80% customers have not faced travelling problem about hero bikes and another 20% customer faced travelling problem of hero bikes.

Chi-Square test:

The chi-square test is defined for the hypothesis:

Null hypothesis H_0: There is no significant difference in the preferable factor and customer satisfaction.

Alternative hypothesis H_1: There is significant difference in the preferable factor and customer satisfaction.

Under the null hypothesis H_0,

Chi square test $=\chi^2=\sum \frac{O_i^2}{E_i} - N \sim \chi 2 = \chi^2_{n-1d.f}$

Preferable Factors	Oi	Ei	Oi^2/Ei
Mileage	72	50	103.68
Pick-Up	56	50	62.72
Price	48	50	46.08
Design	24	50	11.52
	200		224

Cal chi square value$=\sum \frac{O_i^2}{E_i} - N=24$

Tab chi square= 7.815

Therefore,

Cal chi square is greater than Tab chi square

Interpretation: The computed value of chi square is greater than the table value hence the null hypothesis is rejected. So, there is significance difference between the preferable factors and customer satisfaction.

5. Findings:

i. Customers from age between 25-45 are used Hero bike.
ii. Business person as well as government servant, student, farmer preferred tohero bikes.
iii. Number of customers taking purchasing decision through relatives and television media.

iv. From the study of customer satisfactions with special reference to hero bikes, it is found that most of the customer's opinion about bikes is good and excellent. It is observed that 49%customer's opinion is good and 29%customer is excellent about hero bikes.
v. More than 80% of customer said that they do not face any problem while travelling on hero bikes.
vi. There is significant difference between the preferable factors like mileage, pick up, price, design.

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A STATISTICAL STUDY OF CRIME IN MAHARASHTRA STATE

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ABSTRACT: *Crime is one of the most important social problems in the country, affecting public safety, children development & adult socioeconomic status. One of the goals of this paper is to expand the crime statistics. Along with study of violent crimes (like murder, kidnapping, rape) and property crimes (like robbery, burglary, arson, theft). District wise crime data of Maharashtra was collected from government website. In 2014 most of the crimes occurs in Nanded & least in the Nandurbar. In all these crimes, theft crime is mostly happen in all districts. Also, in violent crime that is kidnapping we found that, most of the female victims are in the 12-18 age group kidnapping for marriage and in case of male victims they are kidnapping in 12-16 age groups for other purposes.*

Keywords: *Crime, Crime Statistics, Districts, Types of Crimes, Victims.*

1. Introduction:

Crime in India is very common and happens in many different ways. When you think of crime and criminals you probably think of dangerous looking characters you see played on television or in the movies. But there are lawbreakers like these in real life, too, and you read about their crimes every week in the newspaper**.** India has ranked second in list of countries with highest crime rate, India is also one of the country's that have one of the highest population of the world too, which means a major increase in the number of the crimes. In India as well as in Maharashtra the most of the crimes related are due to theft out of low living situations.

In this study first we discussed about various types of crimes and then discussed purposes behind the crime of kidnapping. In the statistical analysis comparative study is done. District wise crime in Maharashtra state is studied with the help of graphical representations. Area wise proportion of crime is studied with proportional test. Also purpose of kidnapping of male & female in various age groups is studied. Discussion and findings are noted.

2. Statistical Analysis:

The various types of crimes in Maharashtra that are murder, attempt to murder, Rape, kidnapping, Dacoity, Robbery, Burglary, Riots, Thefts, Cheating, Forgery, Arson, Grievous hurt, Dowry deaths, Assault on Women with intent to outrage her, Modesty, Cruelty by Husband or his Relatives, Causing Death by negligence, Extortion, Incidence of rash driving etc.

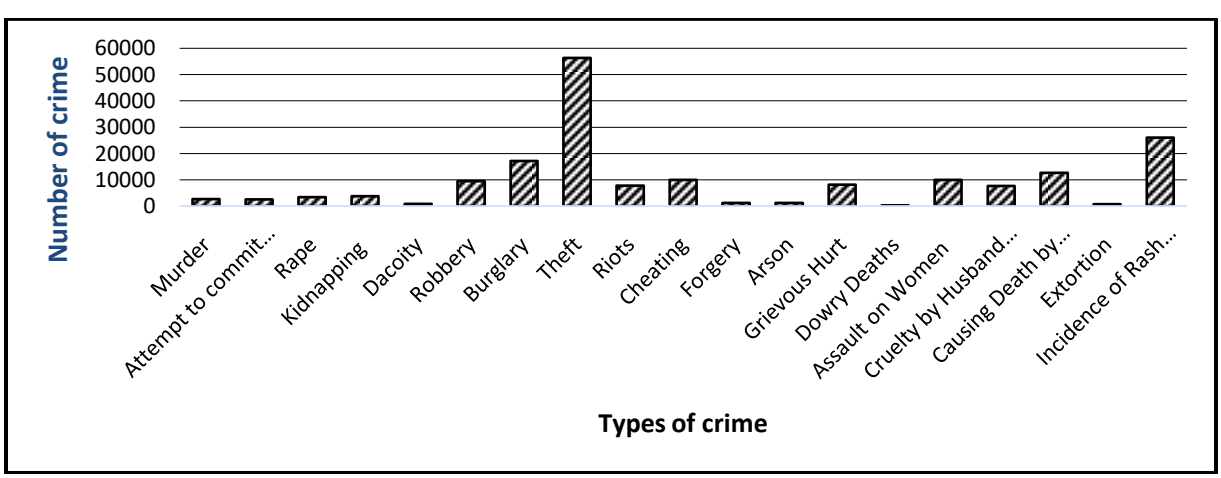

Figure1. Various types of Crimes in Maharashtra

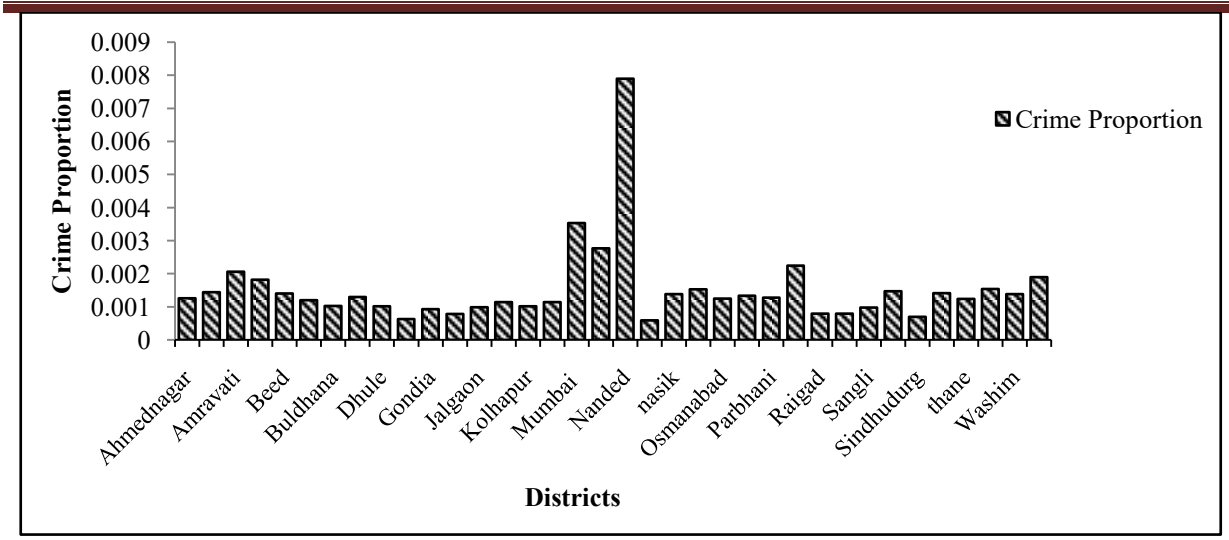

Figure2. District wise crime rate in Maharashtra

Above figure 1 shows, in all various types of crimes the most of repeated crime was theft in all districts and figure 2 shows that, district wise proportion of crimes in Maharashtra. Nanded has ranked first and Mumbai has second in all districts with the highest crime rate. In above figure 2, Satara & Solapur has approximately same crime rate. Hence we check whether the proportion of crime in Satara & Solapur it was same or not by using proportion test.

3. Test Hypothesis:

H_0: The proportion of crime in Satara and Solapur district was same

Vs

H_1: The proportion of crime in Satara and Solapur district was not same

Using R software:

Input:

```
x=c (4435, 6089)
 n=c (3003741, 4317756)
 prop.test(x, n,alternative="two.sided",conf.level=0.95,correct=TRUE)
```

Output:

```
2-sample test for equality of proportions with continuity correction
X-squared = 5.3737, df = 1, p-value = 0.02044
alternative hypothesis: two.sided
sample estimates:
   prop 1     prop 2
0.001476492 0.001410223
```

Conclusion: Here we observe that level of significance 5% is greater than p-value (0.02044), so we reject H_0 that is, the proportion of crime in Satara and Solapur district was not same. Therefore, we conclude that the proportion of crime in Satara district is greater than Solapur district.

Table 1: Study of Violent Crime Kidnapping:

Kidnapping reasons	Age group									
	0-12		12-18		18-30		30-45		45 &above	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
For Adoption	2	1	0	0	0	1	0	0	0	0
For Begging	2	1	0	0	0	0	0	0	0	0
For Illicit Intercourse	1	5	7	65	3	25	3	13	0	4

For Marriage	0	8	0	**637**	0	286	0	139	0	2
For Prostitution	0	0	0	13	0	8	3	1	0	0
For Ransom	7	2	1	1	9	2	8	3	1	0
For Revenge	1	1	3	0	3	3	2	1	1	0
For Sale	0	1	0	0	0	0	0	0	0	0
For Selling Body Parts	0	0	0	0	0	0	0	0	0	0
For Slavery	0	0	0	0	0	0	0	0	0	0
For Unlawful Activity	2	0	7	19	15	28	17	8	0	0
For Murder	5	3	7	8	9	0	3	0	0	0
Others purposes	403	274	**492**	**730**	172	179	115	103	35	8

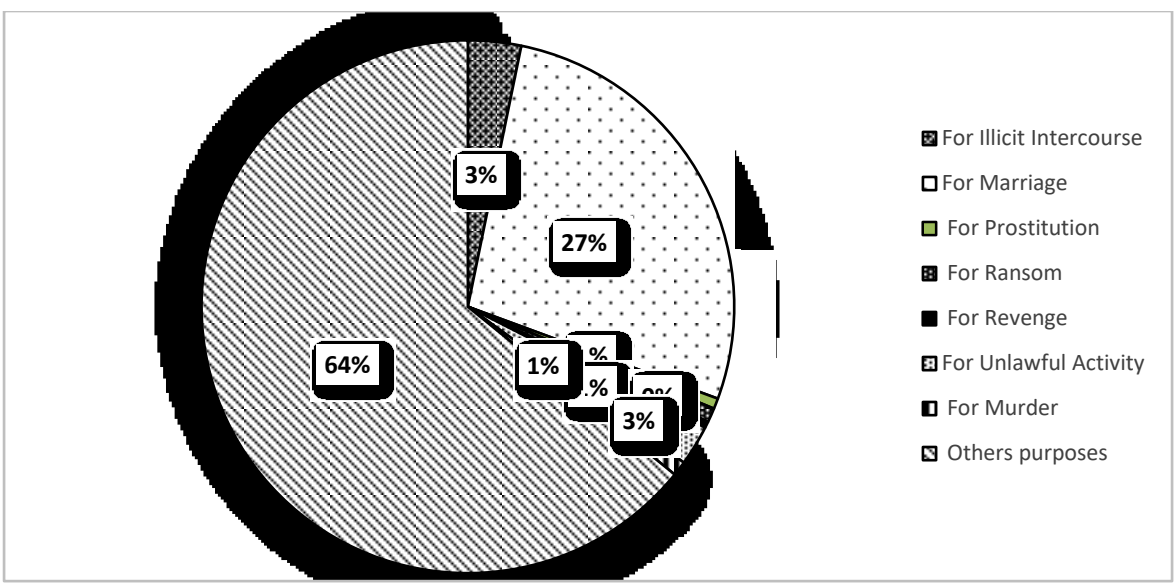

Figure3. Various reasons of kidnapping with their percentage

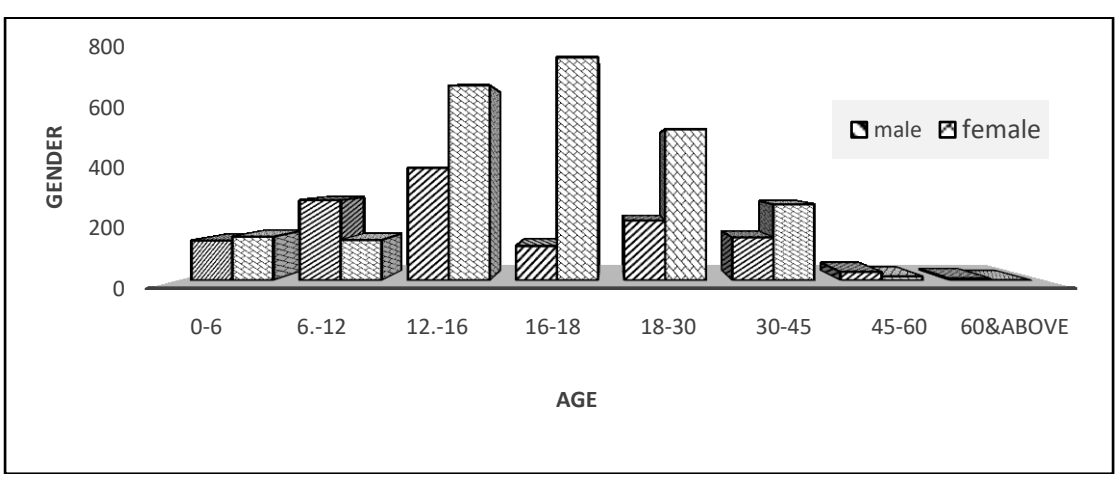

Figure4. Age wise kidnapping of male and female

In figure 3, the 64% kidnapping of male and female is for other purposes, 27% kidnapping of male & female for marriage purposes and there is very less percentage for remaining purposes of kidnapping. In

figure 4, we have seen that most of the male victims are in the 12-16 age groups in kidnapping for other purposes and in the age group of 12-18 the most of the females are kidnapped for marriage purpose.

Pareto chart: Pareto chart named after Italian economist 'Wilfred Pareto'. This type of chart that contains both bars and line graph where individual values are represented in descending order by bars and the cumulative total is represented by the line. When analyzing the data about frequency problems or causes, when there are many causes and we want to focus on the most significant causes then this chart is useful.

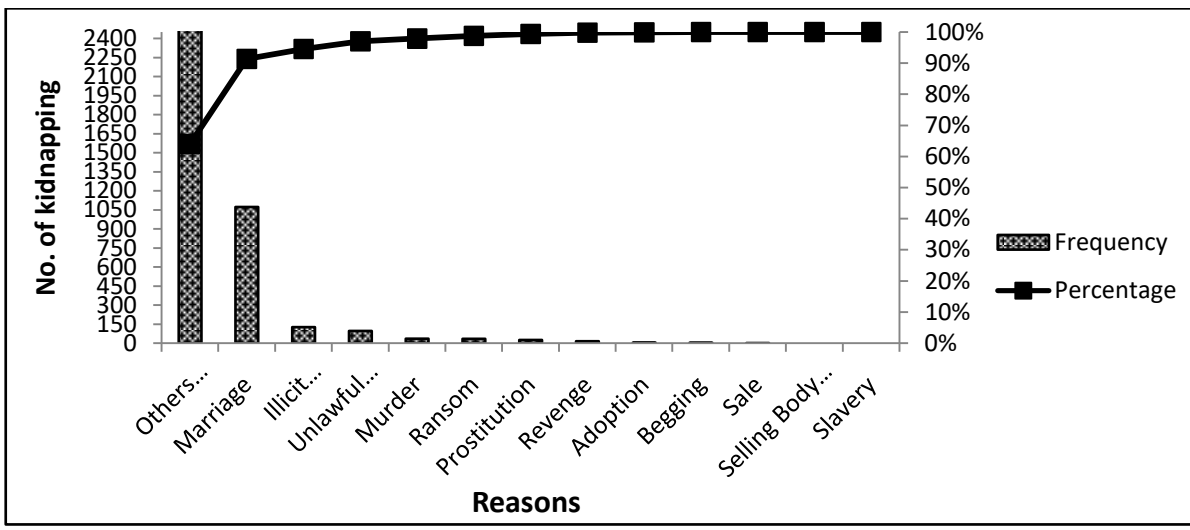

Figure5. Pareto Chart

The above Pareto chart shows the most significant reasons for kidnapping. In this chart bars shows number of kidnapped male and females and the line shows, cumulative total of number of kidnappings. According to this chart, most of the male and females are kidnapped for other purposes and marriage purposes.

4. Findings:

i. Nanded has ranked first and Mumbai has second in all districts with the highest crime rate and Nandurbar has low crime rate.

ii. The proportion of crime in Satara district is greater than Solapur district.

iii. In violent crime that is kidnapping we found that, most of the female victims are in the 12-18 age group was kidnapped for marriage and in case of male victims they kidnapped in 12-16 age groups for other purposes.

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NUPTIALITY STUDY OF RURAL FEMALE POPULATION

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ABSTRACT: *Nuptiality primarily concentrates on age at marriage which is complementary of number of years remained single before marriage. This age at marriage significantly affects fertility to a large extent and hence on population growth. The present study is carried out to develop the Gross Nuptiality Table for Females to understand the marriage probabilities at different ages.*
Women in childbearing age; 15-49 years; from rural area are assessed to study their marital status. Women with their husband, widow and divorced are considered as married. Singulate Mean Age at Marriage (SMAM) by standard method and method given by Hajnal are computed. Further 5 year Nuptiality probabilities are determined.
SMAM by standard method and using alternate method (Hajnal) are same i.e. 22.23 years. Nuptiality probabilities revealed very less probability of marriage of single woman at age 15 years for further 5 years (0.3824) while at other ages i.e. 20, 25, 30, 35, 40 and 45 years it was ≥0.67 for those who are single at that age. SMAM reflects the number of years the burden in sense of social and economical of a girl child on her parents. The Nuptiality table is useful for planning the policies of state and country level governments in sense of welfare of non-marrying women.

Keywords: *Nuptiality, Singulate Mean at Marriage (SMAM), Gross Nuptiality Table*

1. Introduction:

The term 'Nuptiality' used in the sense of study of marriage, primarily concentrates on age at marriage which is complementary of number of years remained single before marriage. This age at marriage significantly affects fertility to a large extent and hence on population growth. The proportions of single at various ages make possible to judge whether marriages are more or less frequent. In India, almost all births occur in marriage, however, variation in age at marriage exist due to socio-economic characteristics like schooling, labour force, health services etc. It is therefore, essential to know the rate at which women of different ages marry before analysis of fertility.

Nuptiality can be studied through a cohort of single females or males. The analysis then consists of observing the change in the proportions of single people over time as the cohort comes to get married. However, marriages may also be studied through the proportions of single persons at each age or age group[1]. Understanding of nuptiality is better achieved by two approaches: a) Singulate Mean Age at Marriage and b) Estimation of Nuptiality probability. This part of the study focuses on application of these both approaches with special reference to women population. However, occurance of marriage is the factor of attrition to develop nuptiality tables. (Life table techniques are extensively used to prepare nuptiality tables.

2. Methodology:

The singulate mean age at marriage (SMAM):

The singulate mean age at marriage (SMAM) is the average length of single life expressed in years among those who marry before age 50 years.

Method of computation:

The singulate mean age at marriage is calculated from the proportions single by age.

$$\text{SMAM} = \frac{15.0 + n\sum_n Sx - K.Sk}{1 - Sk} \quad \text{(i)}$$

Where, the quantity 15.0 is the number of person-years lived in the single state from birth to age 15 by the hypothetical cohort (i.e. it is assumed that no one marries before age 15 years).

n =5 is the age interval

$\sum_n Sx$ is the sum of proportion single from age15 to 49, K is 50 i.e. proportion never marrying at age 50 and will remain so for the rest of the life

Sk is the proportion remaining single between age group 45-49 and 50-54 i.e. $({}_{49}S_{45}+{}_{54}S_{50})/2$

Exact age x starting at 15 years, considered here as the minimum age at marriage. Age 15 is chosen for two reasons. First, beginning at 15 enables one to make international and inter-census comparisons since the previous Census and many censuses in other countries also start at age 15. Second, there are very few marriages before age 15 and trends are therefore overly affected by very minor inconsistencies in the levels[1].

3. Construction of Gross Nuptiality Table:

Column 1) x: Age in years.

Column 2) P_x: Population living at age x.

Column 3) S_x: Number single at age x.

Column 4) s_x: Proportion single at age x. [$s_x = S_x / P_x$]. This calculation should be started from age for which $P_x > S_x$

Column 5) $_5\mathbf{n_X}$: Probability that a single person at age, x will marry during next 5 years i.e. five year nuptiality or marriage probability.

$${}_5n_x = (S_x - S_{x+5})/S_x]$$

Column 6) $\mathbf{l_x}$: Expected number single at age, x in hypothetical cohart of 10000.

$$[\, l_{x+5} = l_x(1 - {}_5n_x)]$$

Column7) $_5\mathbf{N_X}$: Number of marriages in the age group (x, x+5).

$${}_5N_X = \mathbf{l_x}.\,{}_5\mathbf{n_X}$$

Column 8) $_5\mathbf{L_X}$: Number of person years lived as single in the age group (x, x+5)

$${}_5\mathbf{L_X} = \frac{5}{2}(l_x + l_{x+5})$$

Column 9) $\mathbf{T_X}$: Number of years lived as single above age, x.

$\mathbf{T_x} = \sum {}_5L_{5i}$, which is cumulative total from bottom to top.

Column 10) $\mathbf{e_x^0}$: Expected average number of years of single life remaining before marriage at age, x.

$$e_x^0 = T_x/l_x$$

4. Results:

Amongst 5044 total rural female study population, 2862 (56.7%) were in the reproductive age 15-49 years. The mean age of all women in this age was 34.3 years and standard deviation 7.9 years. However of 2862 women 626 (21.9%) were unmarried (Table 1). Thus the mean age at marriage of 2236 women, excluding unmarried women, was 18.5 years with standard deviation 3.6 years. The data of present study revealed that majority of marriages occurred in 18-19 years (13.2%) followed by 17-18 years (12.9%), 16-17 years (11.7%) and 19-20 years (10.8%). Thus about half of women in reproductive age (48.6%) married during 16 to 20 years of age.

Table 1: Marital status of women in reproductive age:

Marital Status	Frequency	%
Unmarried	626	21.9
Divorcee	16	0.6
Married	2082	72.7
Widow	138	4.8
Total	2862	100.0

Further, Singulate Mean Age at Marriage (SMAM) as per (i) was 22.23 years with assistance of Table 2. This inferred that average length as single life, of the woman in reproductive i.e. child bearing age, (those who married before attaining age of 50 years) was 22.23 years. Thus the total number of years of singleness experienced per hundred women of the study population was 2222.77 years, however for whole study population (2236) it was 49701.2 years.

Table 2: Age wise proportion of singleness of women.

Group (i)	Age	Total female population (TFi)	Total never married female population (TNFi)	Proportion single Female U= ${}_nS^f_x$
1	15-20yrs	390	340	0.87179
2	20-25yrs	484	210	0.4338
3	25-30yrs	455	57	0.12527
4	30-35yrs	434	12	0.02765
5	35-40yrs	369	4	0.01084
6	40-45yrs	379	0	0
7	45-50yrs	351	3	0.00855
8*	50-55yrs	302	1	0.00331

* The details of this age group are required for calculation of SMAM and also during development of gross nuptiality table.

Following the alternative procedure suggested by Hajnal[2]1953 referring Table 2:

Person years lived in the single state:

$RS^f_2 = 15 + RS^f_1$ Where 15 is person-years lived single from birth to age 15 years and

$RS^f_1 = 5 \times (U1+---+U7) = 5 \times 1.4779 = 7.3895$

$RS^f_2 = 15 + 7.3895 = 22.3895$

Proportion who ever marries:

Hence the proportion remaining single at age 50:

$RN^f = (U7+U8)/2 = (0.00855+0.00331)/2$

$= 0.01186/2 = 0.00593$

The proportion ever marrying by age 50 (is compliment of proportion remaining single at age 50):

$RM^f = 1.0 - RN^f = 1.0 - 0.00593 = 0.99407$

Number of person years lived by those not marrying:

Total time spent in single state by those who have not married by age 50 is:

$RS^f_3 = 50.0 \times RN^f = 50.0 \times 0.00593 = 0.2965$

$SMAM^f = (RS^f_2 - RS^f_3)/ RM^f = (22.3895 - 0.2965)/0.99407$

$= 22.093/0.99407 = 22.22479302$

The SMAM by (i) and Hanjal procedure are same.

Table 3: Gross Nuptiality for Single Female.

Age in Yrs	P_x	S_x	s_x	${}_5n_x$	lx	${}_5N_x$	${}_5L_x$	T_x	e^0_x
<5	279	279	1.0000	0.0000	100000		500000	2168170	21.68
5-10	298	298	1.0000	0.0000	100000		500000	1668170	16.68
10-15	331	331	1.0000	0.0272	100000		500000	11621703	11.68
15-20	390	340	0.8718	0.3824	100000	39240	404400	668170	6.68
20-25	484	210	0.4339	0.7286	61760	44998	196305	263770	4.27
25-30	455	57	0.1253	0.7895	16762	13234	50725	67465	4.03
30-35	434	12	0.0276	0.6667	3528	2352	11760	16294	4.75
35-40	369	4	0.0108	0.7500	1176	882	9675	4980	4.24
40-45	379	1@	0.0026#	0.6667	294	196	980	1305	4.44
45-50	351	3	0.0085	0.6667	98	65	325	325	3.32

e_x^0 in the above table indicates average number of years expected to live single before marriage at age x for a woman. For example on average an unmarried woman of age 15-20 years is expected to remain single for additional4.27 years before marriage.

@ As there is possibility of someone in each age to be unmarried; calculation is done by assuming 1 unmarried woman in place of 0 i.e. nobody.

${}_5n_x = (S_x - S_{x+5})/S_x$. Since $S_x < S_x+5$, value of ${}_5n_x > 1$. Hence here ${}_5nx=(S_x+5-Sx)/S_x+5$

Column '$_5n_x$' of Table 3 revealed that probability of marriage of a woman, during next 5 years, with age 15 years is comparatively very less than a woman of age 20, 25, 30, 35, 40 and 45 years.
It was observed that 75.2% of women in age group 15-44 years while 78.1% of women in age group of 15-49 years were married. This indicated 20-25% women in marriageable ages remain single due to some or other reason. 32.3% of all married women were in age group of 15-30 years, the first half of the reproductive period.
As per Indian legislature the marriage age of female starts from 18 complete years. Hence (i) may be modified as follows:

$$\text{SMAM} = \frac{18.0 + n\sum_n Sx - K.Sk}{1 - Sk} \qquad \text{(ii)}$$

Where, the quantity 18.0 is the number of person-years lived in the single state from birth to age 18 by the hypothetical cohort.
n =4.5 is the age interval.
$\sum_n Sx$ is the sum of proportion single from age18 to 49.5
K is 49.5 i.e. proportion never marrying at age 49.5 and will remain so for the rest of the life
Sk is the proportion remaining single between age group 45-49.5 and 49.5-54 i.e. $(_{49.5}S_{45} + _{54}S_{49.5})/2$
(Upper age in these age groups are in fact measured in succeeding age group where it is lower limit).

Table 4: Age wise proportion of singleness of women. (modified)

Group (i)	Age	Total female population (TFi)	Total never married female population (TNFi)	Proportion single Female ($_nS^f_x$) or (U)
1	18-22.5	399	265	0.6642
2	22.5-27	467	121	0.2591
3	27-31.5	397	28	0.0705
4	31.5-36	377	5	0.0133
5	36-40.5	358	4	0.0112
6	40.5-45	294	1	0.0034
7	45-49.5	339	3	0.0088
8	49.5-54	274	1	0.0036

This resulted modified SMAM= 22.16yrs. This showed very small change in modified SMAM.
Further modified gross nuptiality table was developed as follows.

5. Construction of Modified Gross Nuptiality Table:

Column 1:x: Age in years.
Column 2:P_x: Population living at age, x.
Column 3:S_x: Number single at age, x.
Column 4:s_x: Proportion single at age, x.
Column 5: $_{4.5}\mathbf{n_X}$: Probability that a single person at age, x will marry during next 4.5 years i.e. 4.5 year nuptiality or marriage probability. $_{4.5}n_x = (S_x - S_{x+4.5})/S_x$
Column 6: $\mathbf{l_x}$: Expected number single at age, x in hypothetical cohart of 10000.
Column7: $_{4.5}\mathbf{N_X}$: Number of marriages in the age group (x, x+4.5).
Column 8: $_{4.5}\mathbf{L_X}$: Number of years expected to be lived as single in the age group (x, x+4.5)
Column 9) $\mathbf{T_X}$: Number of years lived as single above age, x.
Column 10) $\mathbf{e_x^0}$: Expected average number of years as single life before marriage of a woman at age, x.
For these computations the following relations hold good:

$\mathbf{l_x\,(1 - {}_{4.5}n_X)} = l_{x+4.5}$
$\mathbf{l_x .\, {}_{4.5}n_X} = {}_{4.5}N_X$
$_{4.5}\mathbf{L_X} = \mathbf{(4.5/2)}\,(l_x + l_{x+4.5})$
$\mathbf{T_x} = \sum {}_{4.5}L_{4.5i}$, which is cumulative total from bottom to top.
$e_x^0 = T_x/l_x$

Table 5: Modified Gross Nuptiality for Single Female.

Age in yrs	Px	S_x	s_x	${}_{4.5}n_x$	l_x	${}_{4.5}N_x$	${}_{4.5}L_x$	T_x	e_x^0
Less 4.5	253	253	1	-	100000	-	450000	2217225	22.17
4.5-9	265	265	1	-	100000	-	450000	1767225	17.67
9-13.5	296	296	1	0.0068	100000	676	448479	1317225	13.17
13.5-18	306	294	0.9608	0.0986	99324	9793	424915	868746	8.74
18-22.5	399	265	0.6642	0.5434	89527	48649	293411	443831	4.96
22.5-27	467	121	0.2591	0.7686	40878	31419	113258	150420	3.68
27-31.5	397	28	0.0705	0.8214	9459	7770	25083	37162	3.93
31.5-36	377	5	0.0133	0.2000	1689	338	6840	12079	7.15
36-40.5	358	4	0.0112	0.7500	1351	1014	3800	5239	3.88
40.5-45	294	1	0.0034	0.6667	338	225	1015	1439	4.26
45-49.5	339	3	0.0088	0.6667	113	75	340	424	3.75

e_x^0 in the above table indicates that on average an unmarried woman of age 18 years is expected to remain single for more 4.96 years before marriage.

6. Discussion:

Marital status is one of the major elements of population composition. As per Indian culture the present study restrict analysis to single marriages of both men and women from age fifteen to fifty years old. A person who has never married before the age of fifty is regarded as life-long never married in demography (United Nations 1990). In the 1980s, the number of people who married outside the age range from 15 to 50 accounted for less than one in ten thousand of all who entered marriage (Wang 1995)[3].

Singulate mean age at marriage (SMAM) is measure of mean age at marriage obtained from a set of proportions single at different ages. SMAM, the mean age at marriage, is Also known as mean age at effective marriage or average age at marriage[4]. This mean age at effective marriage is an important determinant of the reproductive behavior of the women. Low age at marriage in India is responsible, to a great extent, for high fertility. Mean age at effective marriage for females in India has come up to 21.6years in 2015.[5] The similar SMAM and modified SMAM, 22.4 years and 22.16 years, respectively found in present study. The arithmetic mean age at marriage (18.5 years) was less than the mean age at effective marriage for females.

D. Ghosh[6] brought to notice that 80% of women in age group 15-44 years in 1931 were married. In present study the similar proportion (75% in age 15-44 years and 78% in age 15-49 years) was observed in year 2018. As per demographers the birthrate depends upon the proportion of married women in first half of the reproductive period. This proportion found to be half 32.3% in present study as compared to the proportion observed in 1931(64%). This reduction is indication of slowing the acceleration of population growth.

Majority of marriages occurred in the age 16-20years (48.6%). The mean age of marriage is one of most important indicators in the study of fertility. There are two ways to estimate mean age at marriage - i) Direct method and ii) Indirect method. Direct method asks directly to married about the age at which they have married. Direct question on age at marriage suffers from recall lapse on the part of the respondents as marriage registration is not complete in India. In such situation reporting of the age at marriage suffers from digit preference as such 10, 20, 30 etc. In case of older persons it can grossly underestimate the mean age at marriage. Furthermore, in the younger cohort marriage experience is not complete for all of them. As a result the estimation of mean marriage age suffers from truncation effects. Because of these limitations, a direct question on age at marriage is largely not appropriate to study the trend and pattern in age at marriage in the Indian context. Unlike direct measure which considers married population, the indirect technique takes into account never married population. Based on never-married population, proportion single is calculated by age-groups. Based on proportions single by age-groups, Singulate Mean Age at Marriage (SMAM) is calculated using a method proposed by Hajnal (1953). Hajnal (l953) described proportions single as a tool for studying marriage, which embodies intrinsic advantage over other methods in the absence of marriage registration.[4]

The development of Gross nuptiality tables revealed added advantage over SMAM/modified SMAM. It gives probable more years an unmarried woman at particular age 'x' will remain single. This may help social researches/workers in understanding how the social burdan will release reduce as time passes[4].

Delayed age at marriage has direct impact on population growth as it significantly contributes in reducing fertility.

7. Conclusion:

The nuptiality table found useful in estimating time and incidence of marriage for specific population. However problems are faced during application of lifetable approach to carry out nuptiality study. This is mainly when number of single is high in advancing age groups as compared to its preceding age group while determining the marriage probabilities ($_5n_{x \text{ and } 4.5}n_x$)

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A CASE STUDY OF METHODS OF MONEY TRANSACTION USED BY PEOPLE IN BANKING SECTOR

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ABSTRACT: *The Government wants India to be cashless economy. In our country people used various methods of money transaction like passbook, cheque-book, ATM, online banking. In these methods some are traditional methods and some are cashless methods like ATM, online banking. Before demonetization peoples are mostly used the traditional methods but after demonetization cashless money transaction method is mostly used by peoples.*
In this paper we study the transaction methods in rural and urban areas with age, gender and literacy of people. The method of data collection is primary data survey with a structured questionnaire both rural and urban area.

Keywords: *Traditional methods, Cashless methods, Demonetization, Questionnaire.*

1. Introduction:

The Government wants India to be cashless economy. The move towards a cashless economy is a move towards greater accountability towards the flow of money, reduction in black economy and bringing more people into the banking system.

In our country people used various methods of money transaction like passbook, cheque-book, ATM, online banking. In these methods some are traditional methods and some are cashless methods like ATM, online banking. Before demonetization peoples are mostly used the traditional methods but after demonetization cashless money transaction method is mostly used by peoples. The Indian government with another aim to promote the economy through non-cash transaction has introduced medium such as banking cards, mobile wallet, mobile banking, ATM's. The Digital India programme is a flagship programme of the Government of India with a vision to transform India into a digitally empowered society and knowledge economy. "Faceless, Paperless, Cashless" is one of professed role of Digital India. As part of promoting cashless transactions and converting India into less-cash society, various modes of digital payments are available. In this context, a fact check is essential to ascertain the current status of digital transactions to understand how much our economy has shifted from cash transactions to digital transaction.

2. Motivation of Study:

After demonetization most people are rushing to cashless transaction. Digital transactions bring in better transparency, scalability &Accountability. The government wants India to be cashless economy; the more towards cashless economy is reduction in black money. We want to check that which type of money transaction method is more used by people i.e. cashless or traditional method in rural & urban areas. The other need for the cashless economy is that every transaction that is left unrecorded will now comes into the picture making India one of the fastest growing economies in the world for that transaction method used by peoples are developed.

3. Data Collection and Methodology:

To study of transaction method used by people, we select Urban Area (Vita City) &Rural area (Kamalapur, tal.-khanapur, Dist.-Sangli). The number of population under study of urban area is 666 in that population we take 30% sample of the population is 200 in that population there are 125 male & 75 female. For rural area the total population is 1100 but we take 30% of that population that is 330 in that population there are 175 male and 155 female.

3.1 Proportion Test:

To test in Rural Area & Urban Area Proportion of following terms in male and female are same or not.

1. Proportion Test for awareness of cashless Transaction method in male & female.
2. Proportion Test for awareness of Traditional Transaction method in male & female.
3. Proportion Test for awareness of Transaction method in male & female.

$$\widehat{P} = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2} \text{ and } \widehat{Q} = 1 - \widehat{P}$$

Where,

n_1 = No. of males in particular Area.
n_2 = No. of females in particular Area.
X_1 = No. of awareness of people about Transaction Method in particular Area.
X_2 = No. of not awareness of people about Transaction Method in particular Area.
p_1 = the proportion of Awareness in males about Transaction Method in particular Area.
p_2 = the proportion of awareness in female about Transaction method in particular Area.

3.1.1 Hypothesis:

H_0: $P_1 = P_2$ i.e. there is no significant difference between awareness in male and female about Transaction method.

V/S

H_1: $P_1 \neq P_2$ i.e. there is significant difference between awareness in male and female about Transaction methods.

3.1.2 Test Procedure:

Test Statistic,

$$Z = \frac{p_1 - p_2}{\sqrt{PQ\left(\frac{1}{n_1} - \frac{1}{n_2}\right)}} \sim N(0,1)$$

If cal. Z < tab. Z then accept H_0 otherwise reject it at α % l. o. s.

3.1.3 Rural Area:

Sr. no.	Hypothesis	Cal Z	Tab Z	Proportion values	Conclusion
1	H_0: $p_1=p_2$ Vs H_1: $p_1\neq p_2$	3.8764	1.64	p_1=0.2342 p_2=0.07741	Reject Ho
2		4.2822	1.64	p_1=0.48 p_2=0.2516	Reject Ho
3		2.0910	1.64	p_1=0.1142 p_2=0.0516	Reject Ho

3.1.4 Urban Area:

Sr.no.	Hypothesis	Cal Z	Tab Z	Proportion values	Conclusion
1	H_0: $p_1=p_2$ Vs H_1: $p_1\neq p_2$	5.2199	1.64	p_1=0.3846 p_2 =0.06024	Reject Ho
2		24.4753	1.64	p_1= 0.1367 p_2=0	Reject Ho
3		15.8196	1.64	p_1=0.1362 p_2 =0.1566	Reject Ho

3.2.3 Rural Area:

Sr. no.	Hypothesis	Chi-Sq. Cal	Chi-Sq. Tab	D.F.	Conclusion
1	H_0: A & B are Independent Vs H_1: A & B are not independent	18.80	9.48	4	Reject Ho
2		74.41	42.55	7	Reject Ho
3		21.32	22.36	13	Accept Ho

3.2.4 Urban Area:

Sr. no.	Hypothesis	Chi-Sq. Cal	Chi-Sq. Tab	D.F.	Conclusion
1	H_0: A & B are Independent Vs H_1: A & B are not independent	38.59	16.91	9	Reject Ho
2		13.90	11.07	5	Reject Ho
3		13.03	18.30	10	Accept Ho

4. Limitations of Study:

This study is limited for Urban Area (Vita city) & Rural Area (Kamalapur village). Instead of Urban Area vita city & Rural Area Kamalapur village we can study other cities, villages, states.

5. Conclusion:

i. Proportion of awareness about cashless Transaction in male & female are not same in Rural & Urban Area.
ii. Proportion of awareness about Traditional Transaction in male & female are not same in Rural & Urban Area.
iii. Proportion of awareness about Transaction Method in male & female are not same in Rural & Urban Area.
iv. In both Rural & Urban Area Transaction Method (A) is dependent on Gender (B).
v. In both Rural & Urban Area Transaction Method (A) is dependent on Education (B).
vi. In both Rural & Urban Area Transaction Method (A) is Independent on Annual Income (B).

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A CASE STUDY OF CROPS RICE AND JAWAR IN THE MAHARASHTRA STATE

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ABSTRACT: *Agriculture is one of the most important business in India. Indian economy is mainly depends upon Agricultural business. In Maharashtra, there is high yield of rice and jawar. In this present study we found that, there is positive and strong correlation between area of rice and production of rice (0.818). Also, we found that, there is positive and strong correlation between area of jawar and production of jawar (0.984). The time series analysis was used to predict next (2019 and 2020) year production of rice and jawar in Maharashtra state.*

Keywords: *Agriculture, Rice, Jawar, Correlation, Time series.*

1. Introduction:

In 21st century the production of rice and jawar has been growing steadily all over the India. India, Which Is One Of The Largest Agricultural-Based Economies, Has Made Impressive Strides on the Agricultural Front during the Past Three Decades Much Of The Credit For This Success Should Go To The Several Million Small Farming Families That Form The Backbone Of Indian Agriculture And Indian Economy. India's total geographical area is 329 million hectares. Out of this, 195 million hectare is gross cropped area and 141 million hectare is net sown area. On the other hand, net irrigated area is only 65.3 million hectares. Rest of the land is rain fed. With 35% of the agricultural households having less than 1 acre of land, another 35% holding between 1 and 2.5 acres, and only 30% households with land more than 2.5 acres, the shrinking of agricultural land holdings in India is indeed a cause of concern. Since 1995 -96, the average size land holding has decreased from 1.41 hectares to 1.15 hectares which accounts for the decrease of 30,000 hectares of cultivable land each year.

Agriculture plays a vital role in the Indian economy. Agriculture is the backbone of our country. It includes farming of crops, animal husbandry, pisciculture, agro-forestry etc. Over 58% of rural households primarily depend on agriculture. Agriculture along with fisheries, forestry and other allied sectors contribute around 14% to the overall GDP of our country. The particular weather and soil conditions allow for crops in India uniquely suited to it. Let us take a look at the major crops in India.

The study complete production of rice and jawar in Maharashtra state. The study focuses on the prediction of increases production of rice and jawar in all areas of Maharashtra will be next year. It includes availability increases production, quality of production, farmer's expectations and problems faced by farmers and government. If any at production of rice and jawar in all areas of Maharashtra state.

Varun Kumar Das (2016)[1] studied the Total Factor productivity growth of Jawar and Bajra in India- A comparative Analysis using different methods of TFP computation. He studied the total factor productivity (TFP) growth of jowar and bajra has been estimated using three different methods, viz. Solow index method, Törnqvist-Theil index method and the Malmquist index method.

This study the main objective is growth production of jawar and bajra in india.

2. Materials and methods:

This study is related to the study of crops Rice and jawar in Maharashtra state. The data collected from government website**. The statistical techniques correlation analysis is used to compare area of rice and production of rice and similarly area of jawar and production of jawar and Time series analysis is used to** predict next year (2019 and 2020) production of rice and jawar in Maharashtra state**. The statistical software Excel and SPSS is used for data analysis.**

3. Statistical analysis

3.1 Descriptive Statistics:

Table 1 Descriptive Statistics

Maharashtra	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Jowar	6	1640.19	3566.00	2592.2130	323.59581	792.64463
Rice	6	2183.00	3120.00	2803.8333	138.73510	339.83020

Table-1 gives the descriptive statistics of production of crops namely, Rice and Jawar in Maharashtra state. It shows that, 6 years crops production namely Rice and Jawar. This table also shows that the mean and standard error of production of Rice and Jawar, standard deviation, minimum and maximum production of Rice and Jawar.

3.2 Correlation Analysis:

The pearsons correlation analysis of the two variables is used to determine whether there are relation between two variables. Then types of correlations namely, positively correlation, negatively correlation, no correlation, perfect positive correlation and perfect negative correlation. The hypothesis of correlation is,

1) Rice:

Hypothesis: H_0: There is No Relation Between Area of Rice and Production of Rice.

VS

H_1: There is Relation Between Area of Rice and Production of Rice.

Table 2 Correlations

Model	R	R square	Adjusted R Square	Std. Error of the Estimate
1	0.818	0.670	0.587	1.202

The output of correlation R is show in table-2. The value of correlation is 0.818.Thus reject null hypothesis of relation between area of rice and production of rice. This is strong or perfect positive relation between area of Rice and Production of Rice.

2) Jowar:

Hypothesis: H_0: There is No Relation between Area of Jowar and Production of Jowar.

VS

H_1: There is Relation between Area of Jowar and Production of Jowar

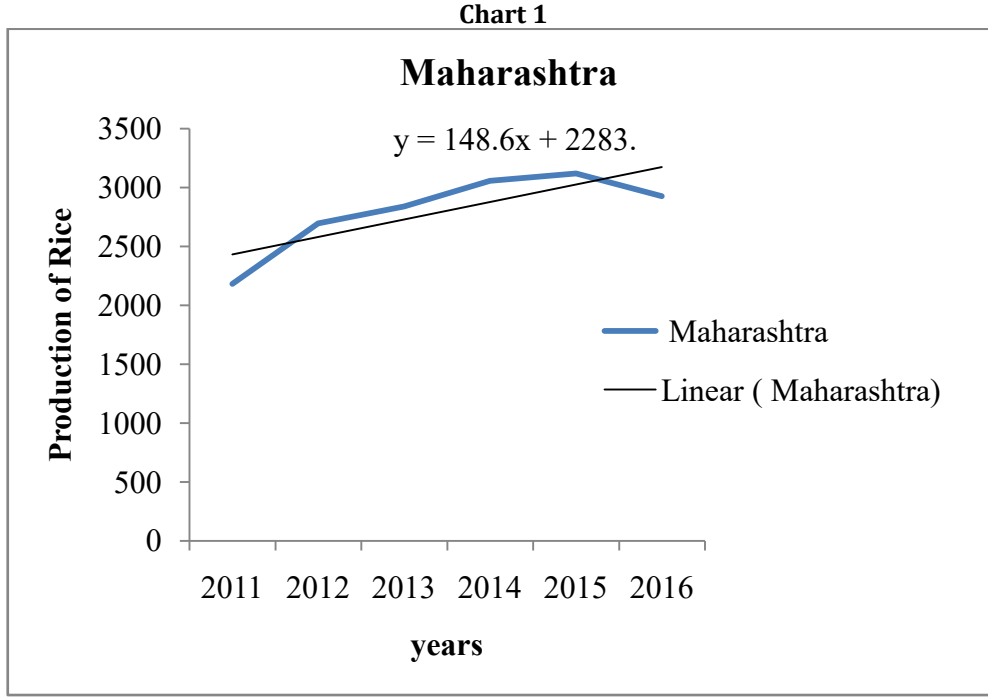
Table 3 Correlations

Model	R	R square	Adjusted R Square	Std. Error of the Estimate
1	0.984	0.884	0.855	0.712

The output of correlation R is show in table-3. The value of correlation is 0.984.Thus reject null hypothesis of relation between area of jawar and production of jawar. This is strong or perfect positive relation between area of jawar and Production of jawar.

3.3 Time Series Analysis:

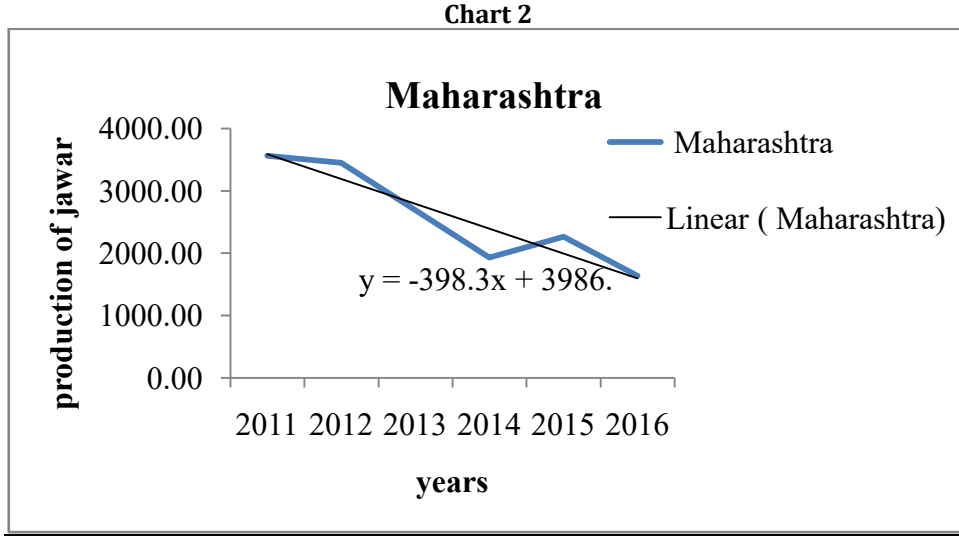
Chart 1



The strength line trend between the time series values of production of rice and year be given by the equation, Y = 148.66x+2283

By using above strength line equation we can estimate the values of production of rice for next year 2019 and 2020 rice of production for Maharashtra will be 3026.29 and 3174.94 thousand tone.

Chart 2



The strength line trend between the time series values of production of jawar and year be given by the equation, Y = -398.3 x + 3986

By using above strength line equation we can estimate the values of production of jawar for next year 2019 and 2020 jawar of production for Maharashtra will be 1994.23 and 1595.87 thousand tones.

4. Conclusion:

The present study A case study of rice and jawar in the Maharashtra state. On the basis of statistical analysis of this study we conclude that, the strong relation between area of rice and production of rice. Similarly the strong relation between areas of jawar and production of jawar. And the time series analysis of this study we conclude that, predict the next year (2019 and 2020) production of rice and

production of jawar increases as compare to present years (2017 to 2018). The results of this study will be help to Department of agriculture cooperation and farmer's welfare and farmers effectively and efficiently.

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VARIOUS FACTORS AFFECTING ON SUCCESS OF SHOPPING MALL IN SOLAPUR CITY

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ABSTRACT: *In 21 century there is increase in shopping malls in tremendous numbers hence large competition among malls to set up and success. To invest national currency in nation the one way is of improvement on shopping mall as per requirement of customers. The success of particular mall is upon the gain of mall or income (budget) and popularity of mall. The data collected from shopping malls of size 400 customers. That is primary type and apply purposive sampling scheme with rule FCFS. From the present study predict budget of customer after knowing ones interest in purchasing items from mall, no of visit, time spent in mall by multiple regression model. The key factors like good service, playing zones for children's, variety of items, parking space, time spent in mall, no. of visits to mall are affecting on success of shopping mall.*

Keywords: *Shopping mall, Customers, Budget, Multiple regressions.*

1. Introduction:

Shopping mall is a place where various local and international brands can sell their products to common man. Indian economy is benefited due to the emergence of supermarkets or airconditioned shopping malls and nowadays there is competition among malls because of increase in shopping mall as well as online shopping scheme. Among online shopping websites Amway, Amazon, Snapdeal etc. are non-Indian websites and due to ease of online shopping scheme, there is craze of shopping from online shopping sites, result of these Non -Indian sites are benefited than our local markets or shopping mall. To overcome this the necessity of developing shopping mall as per requirement of customers. To study this, we select 4 local Indian shopping malls and the various factors affecting on success of shopping mall is observed. Success stands for popularity and budget of particular mall.

The various factors affecting on success of shopping mall are,

1. Locality (specification) of shopping mall
2. Advertisement
3. Availability of parking / Parking facility
4. Entertainment
5. Budget of customers
6. Variety of items / products
7. Cost of items
8. Offers

2. Method and Materials:

Data collected for present study is both primary and secondary type from four malls named D-mart, Reliance, Sai super market, Big bazar. The size of sample is $1/3^{rd}$ (i.e.100) of population that is average number of customers (278-300) per day for each mall and apply purposive sampling scheme with rule FCFS. We selected one main branch of these malls. We did the comparative study of these malls and checked for the factors that are affecting the success of shopping mall used the preplanned questionnaire filled by customers arriving at mall. Due to confidentiality issues raised by mall owners, we collected the data from the customers at the outside of the mall. The success of particular mall is depends upon the gain of mall. On focusing the gain of mall, it depends on no of customers arrives at mall and budget of particular customer.

Methods:

Simple averages, Correlation coefficient, three types of regression: Multiple regression, Binary logistic regression and Nominal logistic regression.

"The success of modern shopping centers relies on a number of critical success factors. These factors include good service, variety in products such as cloths footwear, skin care product, accessories. Also give the better facilities to the customer like food court in mall, plying zone for children, parking for vehicle active marketing." Particular malls.

3. Statistical Analysis:

3.1 Locality (specification) of shopping mall:

D-mart situated at near sector 117, Asara bridge, Jule Solapur has rush of people, vehicles Sai super market and Big bazar located at Satrasta has also rush all time and Reliance supermarket at Degaon Road, Near Laxmi peth, Mari Aai chouk. These places were always rush of peoples.

3.2 Advertisement:

How did customers come to know about latest schemes events and trends of preferred mall they got information from various sources such as newspaper, FM, TV, friends and other. Other includes pamphlet, banner and text messages etc. Following Table gives the consolidated data about this.

Table 1: Percentage customer attracted to mall.

Source of advertisement	News Paper	FM	TV	Friends	Other
No. of customers	71	20	24	130	171
Percentage	0.1775%	0.05%	0.06%	0.325%	0.4275%

From above table1 we conclude that 43% customers came to mall after observing offers from messages (social media), banner and pamphlet.

3.3 Availability of parking / Parking facility:

Among all four malls D-mart has good parking facility, parking available at basement of mall and large area, free passes to regular customer, affordable least cost for parking, also when there is rush to mall at weekends parking area near mall is also available, other three malls has satisfactory parking facilities, but at weekend big problem of parking.

3.4 Budget of customers:

If Budget of customer increases then simultaneously gain of owner increases that is, success of shopping mall. The study mainly focused on increase the budget.

3.4.1 Multiple Regression Analysis:

Regression analysis is mathematical measure of average relationship between two or more variables in terms of original units of the data [2].

3.4.1.1: To quantify the effect of time spent in mall and number of monthly visits to mall on budget, we fitted multiple regression plane using Minitab.

H_0: Time spent in mall and no. of visits to the mall effect on budget.

v\s H_1: Time spent in mall and no. of visits to the mall does not effects on budget.

The Regression equation is, **Budget = 473 + 25.6 time - 75.8 visit**

Here Response variable (y) is budget and covariates are time spent in mall in minutes(X_1) and no. of visits to particular mall in a month(X_2).

Table 2: ANOVA for budget, time and no. of visits.

Source	DF	S S	MS	F	P
Regression	2	374184582	187092291	50.14	$3.84254\times e^{-20}$
Res.error	397	1481397989	3731481	-	-
Total	399	185582571	-	-	-

From above ANOVA, p-value is $3.84254\times e^{-20}$, accept H_0 at 5% level of significance. Conclude that time spent in mall and number of visits to mall effect on budget at 5% level of significance. No. of visits increases budget decrease.

3.4.1.2: To study how budget changes if customers purchase the Cloth-footwear, Accessories, Skin care product and Consumer goods.

H_0: Budget changes with choice of item (material /products) purchased.

v\s H_1: Budget changes with choice of item (material/products) purchased.

The Regression equation is,

Budget = 146 + 1405 Cloth footwear - 109 Accessories + 242 Skin care + 1267 goods

Table 3: ANOVA for budget and purchased product

Source	DF	SS	MS	F	P
Regression	4	222154209	55538552	13.43	0.000
Res. Error	395	1633428362	4135262	-	-
Total	399	1855582571	-	-	-

Here, p-value is 0.00, accept H_0 at 5% level of significance, Budget is most affected by Cloths-footwear and consumer goods.

3.4.2 Binary Logistic Regression:

When Response is categorical instead of using multiple regression one can go for binary logistic regression. To check relationship between choice of products and gender of customer, we use Binary logistic regression.

Link Function: Logit

Table 4: Response information

Gender (Event)	Value	Count
Male	1	255
Female	0	145
Total	-	400

Table 5: Logistic Regression

Predictor	Coefficient	SE Coefficient	Z	P	Odds Ratio
Constant	0.598256	0.312436	1.91	0.056	
Cloth and foot wear	0.542985	0.237956	2.28	0.022	**1.72**
Accessories	-0.035999	0.251233	-0.14	0.886	**0.96**
Skin care cosmetic	-1.01840	0.233215	-4.37	0.000	**0.36**
Goods	0.318012	0.282507	1.13	0.260	**1.37**

From odds ratio, when customer is male then there is 72% chances he purchases Cloths –footwear and 37% of consumer goods also chance of purchasing accessories reduced by 96%& skin care cosmetic by 36%.

3.4.3: Nominal Logistic Regression:

In order to study whether time of customers spent in mall and budget changes, based on whether customer has arrived single, along with a friend or family.

Table 6: Response Information

Variable	Value	Count
Friend=0, Family=1, Single=2	2	231(Reference Event)
	1	90
	0	79
	Total	400

Table 7: Logistic Regression

Predictor	Coefficient	SE Coefficient	Z	P	Odds Ratio
Logit 1:(1/2)					
Constant	-2.46707	0.299742	-8.23	0.000	
Time	0.0074845	0.0033243	2.25	0.024	1.01
Budget	0.0003428	0.0000626	5.47	0.000	1.00
Logit 2:(0/2)					
Constant	-1.84016	0.278171	-6.62	0.000	
Time	0.0050416	0.0033432	1.51	0.132	1.01
Budget	0.0001831	0.0000675	2.71	0.007	1.00

From above table for logit 1, p-value indicates time and budget increases if customer come with family as compared with friends and for logit 2, p-value indicates time and budget increases if customer come with friends as compared to family, but p-value for time in logit 1 is less as compared to logit 2 for budget also hence it indicates budget and Time increases if customer come with family or single. Budget are different when customers comes with family, friend or single.

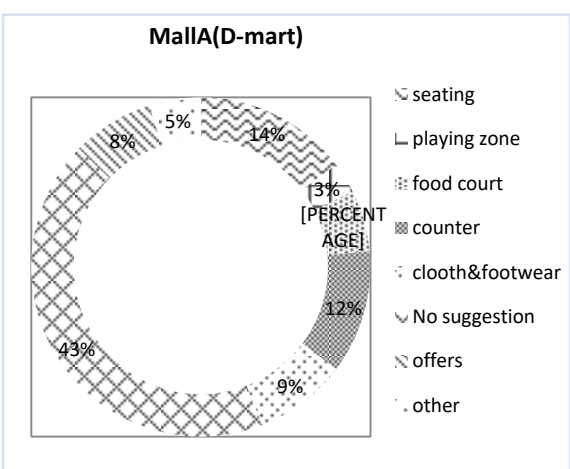
3.5 Suggestions of customers:

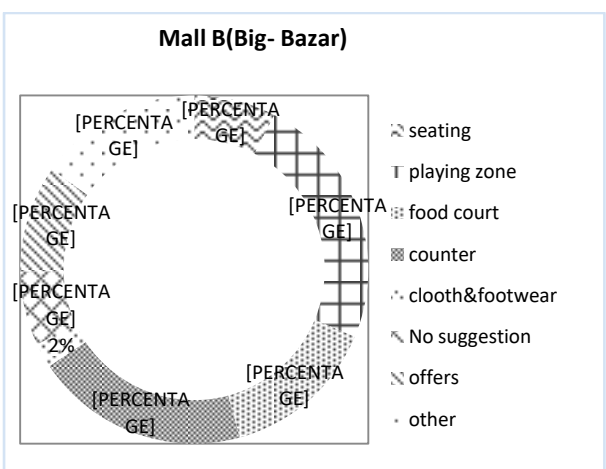
We asked the customers what they would like to suggest for improvement in mall. Following suggestions were obtained.

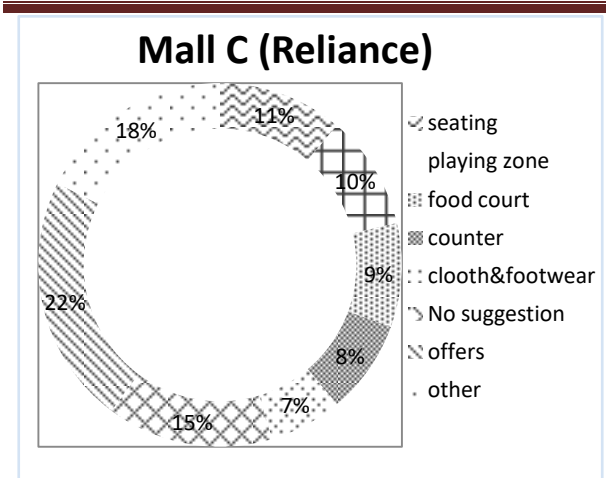
Table 8: For suggestion

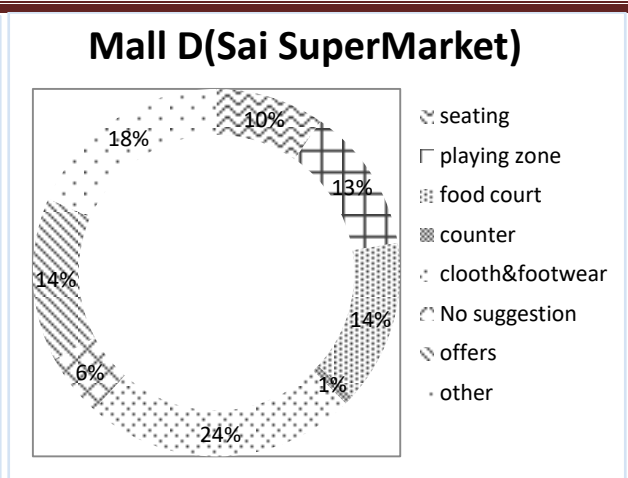
Suggestion	Seating	Playing zone	Food court	Counter	Cloths & footwear	No suggestion	Offers	Other
Mall A (D-mart)	15	3	6	12	9	44	8	5
Mall B (Big - bazar)	10	31	19	26	3	9	13	20
Mall C (Reliance)	12	11	10	9	7	16	24	19
Mall D (Sai super market)	12	16	18	1	30	7	18	23

Graphical Representation of suggestion for shopping malls noted from their feedback:









From above four fig. it indicates that mall A customers are satisfied with it, mall B costumers needs a playing zone and want more cash counters, mall C customers need more discount and fresh vegetables, mall D customers expected playing zone, cloths & Footwear and vegetables.

4. Findings:

Most of customers arrives at shopping mall after observing offers from messages (advertisement on social media), banners and pamphlets. From the present study, predict budget of customer after knowing ones interest in purchasing items from mall and also visits with friends, single or with family using multiple regression model. When customer is male there is 72% chances that he purchase cloths - footwear and 37% of other goods, If required suggestions are applied then increase number of customers ultimately increase budget. D-mart is most successful because possesses the key factors like good service, playing zones for children's, variety of items, parking space.

5. Conclusions:

1. From our study, we conclude that D-mart is most successful as compared to other malls in Solapur city. Because the service, prices of products in d-mart are better than other malls. The approach of customers towards the D-mart is more than other malls.
2. D-mart is successful because it possesses the key factors which attract the customers towards it. It includes parking space, good service, and variety of items, playing zone for children and good co-operation with customers.
3. As compared to other malls, the studied branch of Sai-super market is very less popular mall in Solapur city. This may be because of large number of branches of this mall, that customers of this mall are distributed over its various branches. Moreover, this mall doesn't have sections like cloths and footwear, vegetable, children playing zone, Varity of items etc. The customers are satisfied with such a mall where they can purchase variety of products under the same roof. Maybe because of this aspect, D-mart is successful in attracting larger pool of customers.
4. To increase the budget customer should visits more time for that purpose offers should also increase by mall owners, make places like playing zone, food court. Also parking facility need to be improve.

Limitations:

1. The study is applicable for only four Malls in Solapur city. (D-Mart, Big-Bazaar, Reliance, Sai-Super Market)
2. Sample size is limited.

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STUDY ON ROLE OF MODIFIABLE FACTORS AFFECTING OCCURRENCE OF CORONARY HEART DISEASE

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ABSTRACT: *Coronary Heart Disease (CHD) is a major cause of death. Peoples are aware of having heart problems in the remaining life span, but are they aware from risk factors leading to these problems? The present work was carried out to study role of demographic characters, BP, hypertension, diabetes, diet, physical activities and addiction on occurrence of CHD. For this purpose, a paired matched case-control study was conducted at Aster Aadhar Hospital, Kolhapur. Cox proportional hazard model has used to study which factors affect the time elapsed between start of symptom and the admission to the hospital. This might give us an idea that in older ages the symptoms are obvious to be noticed, but in the young age the span is very less. Modifiable factors like diet, heavy exercise, addiction are affecting occurrence of CHD.*

Keywords: *Coronary Heart Disease (CHD), Case-control study, Modifiable factors.*

1. Introduction:

In today's world, with the growth in Science and Technology, there is also growth in population, urbanization, pollution as well as growth in diseases all over the world. Most of the diseases are known to us. But many times we do not take into account the causes of the particular disease or illness. According to World Health Organization, cardiovascular diseases (CVDs) are the main cause of death; most of the peoples die by CVDs. Coronary heart disease (CHD) is one of cardiovascular disease. Heart diseases are becoming the major cause of mortality in most of the developing countries including India. According to the Centers for Disease Control and Prevention (CDC), more than 370,000 people die from CHD each year in the United States. CHD is the most common type of heart disease; it affects the blood vessels that supply blood and oxygen to heart.

Various risk factors can increase risk of developing CHD, but some of them are modifiable. Hence, it is necessary to be aware of risk factors which are modifiable. For this purpose, present case-control study was conducted at Aster Aadhar Hospital, Kolhapur to study role of modifiable factors affecting on occurrence of CHD.

Rohit V. Ram (April-June 2012) [1] studied association between smoking, smokeless tobacco consumption and occurrence of CHD.

The most important and motivational aspect is to create awareness among people about what are the affecting risk factors in occurrence of CHD and to study effect of various risk factors on time elapsed from start of symptom to admission in hospital.

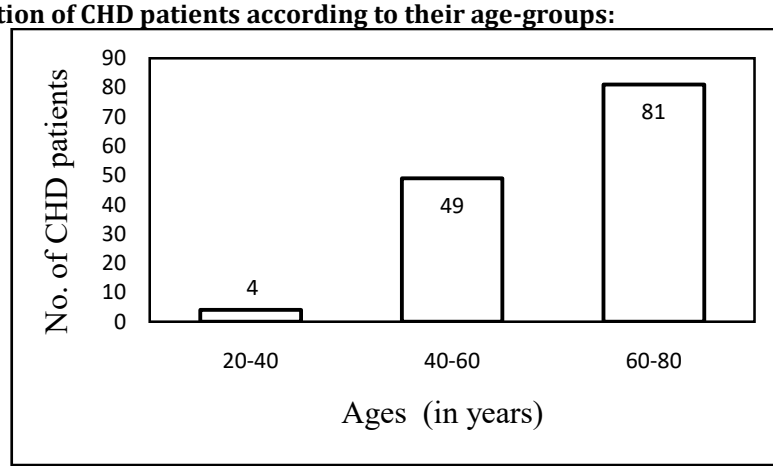
2. Materials and Methods:

A paired matched Case-control study was conducted at Aster Aadhar Hospital, Kolhapur. Before carried out survey, the permission is taken by committee of hospital. The primary data was collected by survey of patients and the secondary data was collected from the record files. The total patients are 268 individuals. Out of them 134 are cases and 134 are controls. Cases are patients who are diagnosed by CHD and controls are patients who have disease other than CHD.

For selection of controls, concept of pair matching was used. We took into account the demographic characteristics such as Age, Gender and confounded these two factors to get the control group which have 1:1 correspondence with the case group people. Hence, case and control are matched with Age ($\pm$1 year) and Gender. Data was analyzed by Minitab and R software. For the Preliminary analysis we have taken account of the Mann-Whitney U test Or Wilcoxon rank sum test, and Fisher exact test.

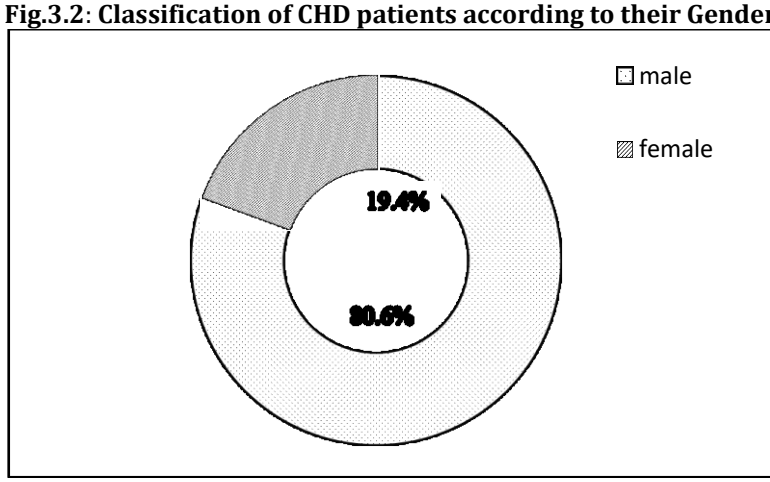
3. Statistical Analysis:

Fig. 3.1: Classification of CHD patients according to their age-groups:



Maximum number of CHD patients belongs to age group above 40 years; hence there can be a risk of CHD after 40 years.

Fig.3.2: Classification of CHD patients according to their Gender:



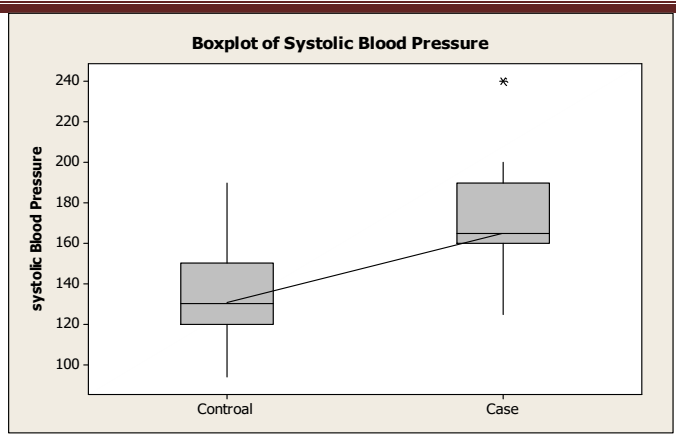
There are 80.6% cases are males and 19.4% cases arefemales. Also, two sample test for equality of proportion gives p-value = 2.2e-16 that means proportions of male and female patients of CHD are not same. Most of the male members have CHD as compare to female.

The data of systolic blood pressure for control group appear to be non-normal with p-value 0.009249. Hence, we have used nonparametric Mann-Whitney U test for equality of medians, we get the following results;

Table 3.1: Comparison of Systolic Blood Pressure

Group	Median	Mean	S.D.	Mann-Whitney U statistics	p-value
Case	165	171.75	25.71	2383.5	**4.403e-09**
Control	130	132.98	18.61		

The difference was significant (P-value =4.403e-09) at 5% level of significance as suggest by Mann-Whitney U test. Thus, systolic blood pressure for case group was significantly higher than that of control group.



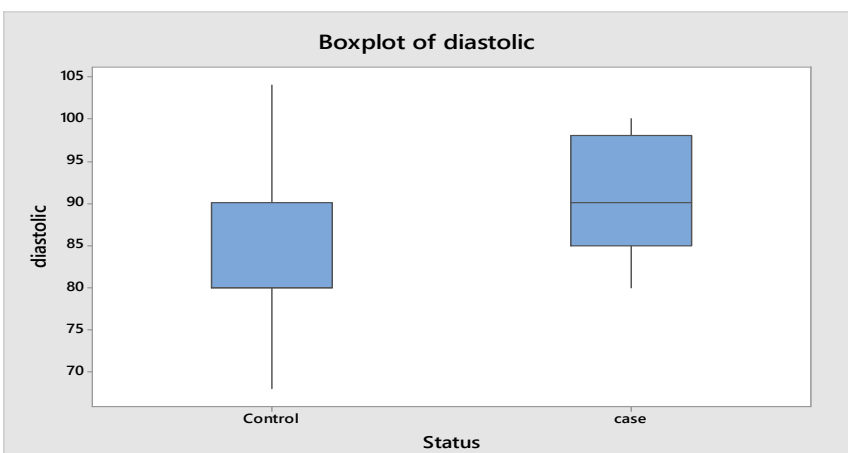
Hence, high systolic blood pressure is a significant factor for the occurrence of Coronary Heart Disease.

The data of diastolic blood pressure for control group appear to be non-normal with p-value less than 0.05. Hence, we have used nonparametric Mann-Whitney U test, we get the following results;

Table 3.2: Diastolic Blood Pressure

Group	Median	Mean	S.D.	Mann-Whitney U statistic	p-value
Cases	90	88.6	9.599	1874	0.001086
Controls	80	82.007	10.6972		

The difference was significant (p-value=0.001086) at 5% level of significance. Thus, diastolic blood pressure for case group was significantly higher than that of control group.



Hence, high diastolic blood pressure is a significant factor for the occurrence of Coronary Heart Disease.

Table 3.3: contingency table for blood group of cases and control

Blood group	O+	O-	A+	A-	B+	AB+	Total
Control	27	3	18	1	23	2	74
Case	31	1	39	2	44	17	134
Total	58	4	57	3	67	19	208

By taking account of Fisher exact test, the blood group appeared to be significant factor (p-value=**0.02791**) at 5% level of significance. The cases of blood group AB+ are 17 which is maximum as compare to control of AB+.

Table 3.4: Comparison of other problems about health of Case and Control group:

Other problems	Status	Cases	Controls	Chi-square test statistic	d.f.	p-value
Hypertension	Yes	80	57	7.2275	1	0.007179
	No	54	77			
Diabetes	Yes	54	42	0.81521	1	0.3666
	No	80	92			

Hypertension (p-value=**0.007179**) significantly affects the occurrence of CHD as observed with chi-square test for independence of attributes. Hypertension is one of the main risk factor in occurrence of CHD.

Table 3.5: Comparison of Case and control group based on diet:

Diet	Status	Case	Control	Wilcoxon test statistic value	p-value
Eggs	Daily	4	2	8966.5	0.9853
	Frequently	49	55		
	Rarely	44	37		
	Never	37	40		
Milk	Daily	62	63	8311	0.2627
	Frequently	19	37		
	Rarely	33	19		
	Never	20	15		
Fruits	Daily	62	37	10397	0.01714
	Frequently	40	61		
	Rarely	32	36		
Cereals	Daily	92	126	6717	1.319*e^-07
	Frequently	41	7		
	Rarely	1	1		
Pulses	Daily	35	58	6717	1.319*e^-07
	Frequently	91	69		
	Rarely	8	7		
Non-veg	Daily	6	4	8908	0.9084
	Frequently	49	53		
	Rarely	36	35		
	Never	43	42		

In Table 3.5, it can be seen that the dietary contents such as Fruits, Cereals and Pulses are significantly affecting the occurrence of CHD. Cereals and pulses content should be increased in the daily diet of every individual because the people in the Case group are low cereals and pulses eaters. Daily intake of food in case group is maximum as compare to control group.

Table 3.6: Comparison of Case and Control group based on Exercise: -

Exercise	Status	Case	Control	Wilcoxon statistic	p-value
Walking	Daily	76	89	8043	0.08522
	Frequently	2	5		
	Rarely	6	3		
	Never	50	37		
Jogging	Daily	11	2	9581	0.01071
	Rarely	123	132		
Yoga	Daily	11	9	9113	0.6558
	Rarely	1	1		
	Never	122	124		

Jogging has significant effect on the occurrence of CHD. Also, we can see that out of 13 jogging people, 11 are under Case group.

Table 3.7: Comparison of Case and Control group based on Habits:

Habits	Status	Case	Control	Wilcoxon statistic	p-value
Smoking	Daily	19	7	9863	0.01445
	Frequently	1	1		
	Rarely	3	2		
	Never	111	124		
Drinking	Daily	9	5	9768.5	0.04444
	Frequently	5	4		
	Rarely	12	5		
	Never	108	120		
Tobacco	Daily	33	17	10190	0.007613
	Frequently	3	2		
	Rarely	2	1		
	Never	96	114		

Smoking, drinking and tobacco consumption has significant effect (p-value<0.05) on occurrence of CHD. Among total people having these habits, majority of them are belonging to the case group; such habits can lead to CHD.

Cox-proportional Hazard Model:

The Cox proportional-hazards model (Cox, 1972) is a regression model and it investigate association between the time to event and one or more covariates. This model is used to study which factors affect the time elapsed between start of symptom and the admission to the hospital. The model is fitted by using survival and survminer packages in R. The assumption of proportional hazard is checked by using residuals in R.

Here, T: Time (in days) elapsed between start of symptoms to admission to the Hospital.

Indicator variables are:

$$\text{Jogging} = \begin{cases} 1, & \text{if patient did jogging daily} \\ 0, & \text{O. w.} \end{cases}$$

$$\text{Tobacco “X”} = \begin{cases} 1, & \text{ifpatient has using tobacco to level "X"} \\ 0, & \text{O. w.} \end{cases}$$

Where, X ϵ {Daily, Frequently, Rarely}

Table 3.8: Baseline Category for different Predictors

Covariates	Baseline Category
Jogging	Never
Tobacco	Never

Table 3.9: Fitted parameters of Cox Model

Covariates	Coefficient	Exp(coef.)	S.E.(coef)	Z	p-value
Age	-0.04091	0.95992	0.01243	-3.291	0.000997
Gender	0.65429	1.92377	0.2714	2.411	0.015917
HB(Hemoglobin(g/dl))	0.16096	1.17464	0.0742	2.169	0.030059
Jogging_Daily	1.38538	3.99636	0.48742	2.842	0.004479
Tobacco_Daily	0.49484	1.64024	0.24699	2.004	0.045123
Tobacco_Frequently	0.88682	2.42741	0.74072	1.197	0.231212
Tobacco_Rarely	-0.50292	0.60477	0.74065	-0.679	0.497124

Table 3.10: Baseline Hazard ($h_0(t)$)

Time	Hazard	Time	Hazard	Time	Hazard
1	0.050299	62	0.509526	545	1.726516
2	0.06812	90	0.569845	548	1.794887
3	0.095762	120	0.676602	730	2.210214
8	0.124781	150	0.721538	1095	3.178139
10	0.144989	180	0.924394	1780	3.856499
12	0.155348	210	0.953325	2555	5.018509
15	0.210819	240	0.983191	2888	5.724243
20	0.235441	305	1.013925	4380	7.490997
30	0.343654	365	1.662874		

The proposed model is,

$h(t) = h_0(t) \times \exp\{(-0.04091 \times \text{Age}) + (0.65429 \times \text{Gender}) + (0.16096 \times \text{HB}) + (1.38 \times \text{Jogging\_Daily}) + (0.49484 \times \text{Tobacco\_ Daily}) + (0.88682 \times \text{Tobacco\_Frequently}) - (0.50292 \times \text{Tobacco\_Rarely})\}$

Where, $h_0(t)$ is baseline hazard function.

In table 3.9, we see that age, HB, Daily Jogging, Daily Tobacco consumption affected the time span. Hazard rate of male is 0.95992 times hazard rate of female; the time span of female lesser as compared to males in case group which gives that females show later symptoms than males. Hazard rate of daily tobacco chewer is 1.64024 times hazard rate of people never habit of tobacco. Time span of daily tobacco chewer lesser which gives a crude view that tobacco using damages the health slowly and after particular time it suddenly gives rise to the CHD.

4. Conclusions:

a) Risk of CHD after age 40 years and males have high risk of CHD than females.

b) As the systolic as well as diastolic BP of the Case group people was higher than that of the control group which is seen to be significant factor to the occurrence of CHD. Hypertension is seen to be significant factor; hence people must take precautionary measures in order to maintain the low stress level.

c) The cereals contain large quantity of carbohydrates can reduces the blood cholesterol level in body which lead to reduce heart diseases. High protein content in pulses which may reduce the occurrence of CHD at a very low level. A regular intake of food is good for health but high level of pesticides in fruits may be harmful to health and it can lead to development of diseases. Habits such as smoking, drinking and tobacco must be completely avoided for a healthy life.

d) Jogging is significant factor in occurrence of CHD hence, peoples have to do effective exercises like walking and yoga to avoid physical exertion.

e) Females show later symptoms of CHD than male. As Age increases, the time span between the start of symptoms and admission to the hospital is also increased. That means older ages the symptoms are obvious to be noticed, but in the young age the span is very less that means that the people can have a sudden shock.

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SAMPLE SIZE CALCULATION FOR SUPERIORITY PARALLEL GROUP CLINICAL TRIAL

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ABSTRACT: *Canadian ecologists Mudge et. al (2012a, 2012b) has suggested an optimal α approach by minimizing the weighted sum of error rates in which weights are the relative costs of the type I and type II errors instead of fixing the value of type I error (α) and minimizing type II error (β). In this paper, we propose a sample size calculation procedure for superiority parallel group clinical trial by minimizing the weighted sum of errors for normal response variable, when variance is known and unknown. Note that Flight and Julious (2016b) procedure for calculations of sample size for superiority trials is a particular case of our proposed procedure.*

Keywords: *Sample size; Type I error; Type II error; Power; Optimal α .*

1. Introduction

Sample size issues are important for the planning of clinical trial. When planning a trial, an important step is the calculation of the minimum sample size required to meet the given objectives of the study. To compare two treatments, Julious (2004) has given an overview of sample size calculations for parallel group and cross-over trials with normal data. Flight and Julious (2016a) were highlighted the general components required to estimate the sample size of a clinical trial. There are mainly four types of trials for hypothesis testing: 1) Superiority trials, 2) Non-inferiority trials, 3) Equivalent trials and 4) Bioequivalence trials. Flight and Julious (2016b) has highlighted practical guide for sample size calculation for superiority parallel group clinical trials, where the primary endpoint can be assumed to be normally distributed

In parallel group design, patients are assigned at random to the two treatments to form two treatment groups. It is hoped at the end of trial that the two groups are the same in all respects other than the treatment received. In cross over design, each patient receives both the treatments. But the order that patients receive the treatment is randomized. Here the assumption is that prior to starting the second treatment, all patients return to baseline and that the order in which patients receive treatment does not affect their response to treatment.

The null hypothesis significance tests (NHST) can be used as decision-making tools. The goal of these tests should be to provide us with conclusions in which we have the highest possible confidence. In NHST, there are two types of errors, type I error and type II error. A type I error (α) is the probability of rejecting the null hypothesis when it is true. A type II error (β) is the probability of accepting the null hypothesis when this is false. The choice of a α level will determine the probability of a type II error for a study with a given sample size and critical effect size. Decreasing the probability of type I error increases the probability of type II error and vice versa. So it is very important to choose appropriate value of α and β. Usually we set α as fixed and chose decision criterion or critical region in order to minimize the probability of type II error and hence maximize the power.

Mudge et. al (2012a) suggested that, instead of fixing the type I error (α) rate and minimizing the type II error rate, choose an optimal value for the type I error rate by minimizing the weighted sum of the error rates in which the weights are the relative costs of type I error and type II errors. The optimal α level has the flexibility to minimize either combined probability of type I error and type II error or the relative cost of errors. Mudge et al (2012b) suggested that sample size can also be determined by minimizing the weighted sum of error rates. And it is independent of both error rates. Grieve (2015) has proposed the sample sizing based on minimizing the weighted sum of errors for single normal response variable

In a sequential clinical trial some information is collected and is examined for it to be good enough to terminate the experiment and take a decision or continue to gather additional information. Such procedures are used to reduce the cost/ time under certain level of precision. For the details see Ghosh et. al.(1997).Here we propose a rule for calculating sample size for superiority parallel group clinical trials by minimizing the weighted sum of errors for normal response variables with known and unknown variances. Our proposed rule is a generalization of Flight and Julious (2016b) procedure for superiority trials.

2. Superiority Clinical Trials with Known Variance Case.

In superiority trial, null and alternative hypotheses are H_0: The two treatments are not different with respect to the mean response $\mu_A = \mu_B$. H_1: The two treatments are different with respect to the mean response $\mu_A \neq \mu_B$. That is we want to test that the two means are equal, against an alternative that they differ by an amount d, where d is effect size. The allocation ratio (r) is such that the number of participants on treatment B is r times the number of participants on treatment A, that is $n_B = r\, n_A$. Note that n ($n = n_A + n_B$) is minimized when r =1. The sample size per arm can be given by following formula

$$n_A = \frac{(r+1)(Z_{1-\beta} + Z_{1-\alpha/2})^2 \sigma^2}{rd^2}, \qquad (2.1)$$

where $Z_{1-\alpha/2}$ denote the (1-α/2) percentage point of a standard normal distribution and $Z_{1-\beta}$ denote the (1-β) percentage point of the standard normal distribution

2.A) Optimal α:

An optimal α can be calculated for any hypothesis test. Optimal α is the significance level that is associated with the minimum average of α and β and weighting this average by the a priori relative probabilities of the null and alternative hypothesis or relative cost of type I and type II errors. From equation (2.1) we can write

$$(Z_{1-\beta} + Z_{1-\alpha/2}) = \sqrt{\frac{n_A r}{(r+1)}}\frac{d}{\sigma} = \theta. \quad \text{(say).}$$

$$Z_{1-\beta} = \theta - Z_{1-\alpha/2} = \theta + Z_{\alpha/2}. \qquad (2.2)$$

Thus $$\beta = 1 - \phi(\theta + Z_{\alpha/2}) \qquad (2.3)$$

where φ is the cumulative density function of N (0,1). Optimal α level should be obtained by minimizing the cost weighted average of α and β instead of minimizing the combined probabilities of type I and type II error. Let w be either the relative prior probability of the null and alternative hypothesis being true or the relative cost of the errors. For detail refer to Mugde et.al (2012a). For a given value of w, weighted sum of the probabilities of type I and type II errors is given by ψ = wα+β/w+1. From equation (2.3)

$$\psi = \frac{w\alpha + 1 - \phi(\theta + Z_{\alpha/2})}{w+1}. \qquad (2.4)$$

For given value of w, ψ is function of α alone. Now we obtain the value of α which minimizes ψ, for this solving the equation $d\psi/d\alpha = 0$, we get

$$\frac{w}{2} = \frac{\varphi(\theta + Z_{\alpha/2})}{\varphi(Z_{\alpha/2})}, \qquad (2.5)$$

where $\varphi(x) = \sqrt{\frac{1}{2\pi}} e^{-\frac{x^2}{2}}$.

$$\Rightarrow \qquad Z_{\alpha/2} = \frac{-\ln(w/2)}{\theta} - \frac{\theta}{2}. \qquad (2.6)$$

Hence we have,

$$\alpha = 2\phi\left(\frac{-\ln(w/2)}{\theta} - \frac{\theta}{2}\right). \qquad (2.7)$$

Now the corresponding value of the probability of type II error is $\beta = 1 - \phi\left(\frac{-\ln(w/2)}{\theta} + \frac{\theta}{2}\right) = \phi\left(\frac{\ln(w/2)}{\theta} - \frac{\theta}{2}\right)$. Thus the minimum weighted sum is $\psi = 2w\phi\left(\frac{-\ln(w/2)}{\theta} - \frac{\theta}{2}\right) + \phi\left(\frac{\ln(w/2)}{\theta} - \frac{\theta}{2}\right) \Big/ w+1$. From above equation we say that ψ is function

of $\theta = \sqrt{\frac{n_A r}{(r+1)}}\frac{d}{\sigma}$, so it can be written as $\psi(w, \theta)$. Note that $\psi(w,\theta) = \psi(w^{-1},\theta)$. If w =1 that is equal cost case, the minimum weighted sum occurs when α = β.

2.B) Calculation for w:

Suppose the experiment has been designed for fixed α_0 and power $(1-\beta_0)$.The sample size has been chosen from equation (2.1). Usually we set $\alpha_0 = 0.05$ and $\beta_0 = 0.1$ or 0.2, implying that type I error is more costly than type II error. Substituting equation (2.2) in equation (2.5) we get $w = \frac{2\varphi(Z_{1-\beta_0})}{\varphi(Z_{\alpha_o/2})}$. When we have 10% type II error and 5% two-sided type I error rate, the value of w is $\frac{2\varphi(1.282)}{\varphi(-1.96)} = 6.002555$. This implying that the cost of a type I error is six times that of a type II error. Note that w is independent of $\delta = d/\sigma$.That is w remain constant whatever may be the value of δ.

As θ → 0, ψ(w, θ) → 2w/(w+1) which implies that ψ(w, θ) cannot be determined for given w. Suppose we want to control the minimum weighted sum of errors at a maximum of $\psi_o(w, \theta)$. In the following we propose a calculation procedure of sample size.

2.C) Calculation of sample size:

For given $\psi_o(w,\theta)$, w, d, r and σ, the sample size (n_A) is obtained by following rule.

Stop for the first time, if

$$2w\phi\left(\frac{-\ln(w/2)}{\sqrt{\frac{n_A r}{(r+1)}}\frac{d}{\sigma}} - \frac{1}{2}\sqrt{\frac{n_A r}{(r+1)}}\frac{d}{\sigma}\right) + \phi\left(\frac{\ln(w/2)}{\sqrt{\frac{n_A r}{(r+1)}}\frac{d}{\sigma}} - \frac{1}{2}\sqrt{\frac{n_A r}{(r+1)}}\frac{d}{\sigma}\right) < \psi_o(w,\theta)(w+1). \qquad (2.8)$$

Since w is finite and $\psi_o(w,\theta)$ is also finite, the rule (2.8) is closed.

2.D) Computational study:

In this section, we compute sample sizes n_A for one group in a parallel group study for different values of standardized differences (δ = d/σ), allocation ratios (r) and weighted sum of errors $\psi_o(w,\theta)$. Taking 10% type II error and 5% two-sided type I error rate the value of w is $w = \frac{2\varphi(1.282)}{\varphi(-1.96)} = 6.002555$, following results are obtained using (2.8).

Table 2.1

Sample sizes for one group, n_A in a parallel group study for different values of standardized differences $(\delta = d/\sigma)$, allocation ratios and $\psi_0(w,\theta)$ =0.05714073				
	Allocation ratio(r)			
δ	1	2	3	4
0.05	8407	6306	5605	5255
0.1	2102	1577	1402	1314
0.15	935	701	623	584
0.2	526	395	351	329
0.25	337	353	225	211
0.3	234	176	156	146
0.35	172	129	115	108
0.4	132	99	88	83

0.45	104	78	70	65
0.5	85	64	57	53
0.55	70	53	47	44
0.6	59	44	39	37
0.65	50	38	34	32
0.7	43	33	29	27
0.75	38	29	25	24
0.80	33	25	22	21
0.85	30	22	20	19
0.90	26	20	18	17
0.95	24	18	16	15
1.00	22	16	15	14

3. Superiority Clinical Trials with Unknown Variance Case.

In clinical trial when data have been collected and used for analysis, it is usually the case that the population variance (σ^2) is considered to be unknown and a sample variance estimate is used. So in such cases we have to use t-statistics instead of Z-statistics. Thus, we have

$$\beta = 1 - F_v\left(t_{1-\frac{\alpha}{2},\nu},\ \nu,\ \theta\right) \qquad (3.1)$$

where $F(\bullet, v, \theta)$ is defined as the cumulative density function of a non-central t distribution with v = $n_A(r+1)-2$ degree of freedom and non-centrality parameter θ = $\sqrt{\frac{rn_A d^2}{(r+1)\sigma^2}}$. and $t_{1-\frac{\alpha}{2},\nu}$ is the critical value of a t distribution with v degree of freedom. The weighted sum of error is $\psi = \frac{w\alpha + 1 - F_v\left(t_{1-\frac{\alpha}{2},v}, v, \theta\right)}{w+1}$. Then value of α is obtained by solving the equation $\frac{d\psi}{d\alpha} = 0$, we get,

$$w - \frac{f_v\left(t_{1-\frac{\alpha}{2},v}, v, \theta\right)}{f_v\left(t_{1-\frac{\alpha}{2},v}, v, 0\right)\left(\frac{1}{2}\right)} = 0, \qquad (3.2)$$

where $f_v(t, v, \theta)$ is the density function of a non-central t distribution with v degree of freedom and $f_v(t, v, 0)$ is the density function of a central t distribution with v degree of freedom. From equation (3.2), α is obtained called optimal α and is denoted by $\alpha_{optimal}$.

3.A) Calculation for w:

Suppose the experiment has been designed for fixed α_0 and power $(1-\beta_0)$. Usually we set $\alpha_0 = 0.05$ and $\beta_0 = 0.1$. Sample size has been chosen from equation (3.1). Let it will be n_{A_o} . Then the value of w is obtained by following equation

$$w - 2\left(\frac{f_{v_0}\left(t_{1-\frac{\alpha_0}{2},v_0}, v_0, \theta_0\right)}{f_{v_0}\left(t_{1-\frac{\alpha_0}{2},v_0}, v_0, 0\right)}\right) = 0, \qquad (3.3)$$

where $v_0 = n_{A_o}(r+1)-2$ and $\theta_0 = \sqrt{\frac{rn_{A_0}d^2}{(r+1)\sigma^2}}$. Using the equation (3.3), the values of w are obtained for different δ and r as in the following Table 3.1

Table 3.1

For different values of δ and r, the values of w are obtained by equation (3.3)				
δ\ r	1	2	3	4
0.05	6.006569	6.0048	6.005187	6.004165
0.1	6.006828	6.007669	6.004267	6.006703
0.15	6.015194	5.999259	5.996059	5.993526
0.20	6.007886	6.011253	6.009513	6.007376
0.25	6.004228	6.021901	6.006768	5.995997
0.30	6.017648	6.025234	5.967765	5.973654
0.35	6.025842	5.987429	6.030783	5.994977
0.40	,6.012318	5.962174	5.923628	5.972042
0.45	6.06708	6.00311	6.014376	5.919213
0.50	5.973875	6.045023	5.983966	6.060309
0.55	6.042127	6.067777	6.054155	6.037621
0.60	6.028469	5.986612	5.934805	6.066445

and so on.

3.B) Procedure for sample size:

Suppose we wish to control the minimum weighted sum of errors not to be more than $\psi_0(w,\theta)$. For given $\psi_0(w,\theta)$, w, d, r and σ, the sample size (n_A) is obtained by following rule.

Stop for the first time, if

$$w\alpha_{optimal} + F_v\left(t_{1-\alpha_{optimal}/2,v}, \theta\right) < \psi_0(w,\theta)\,(w+1), \qquad (3.4)$$

where $\alpha_{optimal}$ is obtained by equation (3.2). Since w is finite and $\psi_0(w,\theta)$ is also finite, the rule (3.4) is closed.

3.C) Computational study:

In this section, we compute sample sizes n_A for one group in a parallel group study for different values of standardized differences $(\delta = d/\sigma)$,allocation ratios(r) and weighted sum of errors $(\psi_0(w,\theta))$. Taking different values of w from Table 3.1, following results are obtained

Table 3.2

Sample sizes for one group, n_A in a parallel group study for different values of standardized differences $(\delta = d/\sigma)$, w, allocation ratios and $\psi_0(w,\theta)$ = 0.05714073 Allocation ratio(r)				
δ	1	2	3	4
0.05	8406	6306	5605	5254
0.1	2103	1577	1402	1314
0.15	935	702	624	585
0.2	527	395	351	329
0.25	338	254	225	211
0.3	235	176	157	147
0.35	173	130	115	108
0.4	133	100	89	83

0.45	105	79	70	66
0.5	86	64	57	53
0.55	71	53	47	44
0.6	60	45	40	37
0.65	51	38	34	32
0.7	44	33	30	28
0.75	39	29	26	24
0.8	34	26	23	21
0.85	31	23	20	19
0.9	27	21	18	17
0.95	25	19	17	15
1.00	23	17	15	14

4) Worked example:

Consider the example given by Julious (2004). Suppose an investigator wishes to design a hypertension trial with equal allocation (that is r=1) between groups where the clinical effect of interest is a reduction in blood pressure compared to control of 10 mmHg (d). The expected standard deviation in the population in which the trial is to be undertaken is 40 mmHg (σ). Therefore, the standardized difference (δ) is 10/40 = 0.25. Suppose we wish to control the sum of errors $\psi_0(w,\theta)$ to be no more than 0.05. Then sample size is n_A= 366 by stopping rule (2.8) with w = 6.002555 and n_A= 367 by stopping rule (3.4) with w= 6.004228.

Remark 1)) Sample sizes of the proposed rule with known variance case are slightly less than unknown variance case because of w.

Remark 2) In both cases, sample sizes of the proposed rule are nearly same as that of Flight and Julious (2016b) for $\psi_0(w,\theta)$ =0.05714073 that is Tables (2.1) and (3.2)

Remark 2) From above Tables (2.1) and (3.1), it is clear that as δ and allocation ratio (r) increases, sample size decreases.

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STUDY OF EFFECT OF CASHLESS TRANSACTIONS

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ABSTRACT: *Cashless means we take anything without giving cash. Cashless economy is an economic system in which there is little or very low cash flow in a society and goods and services are bought and paid through electronic media. For this project the primary data is collected from 200 respondents in vita city. We study that how many people use Cashless transaction. We use the proportion tests to find out the proportion about ATM user, opinion about digital city and also use the chi-square tests to check, the dependency between education and Cashless transaction.*

Keywords: *Cashless, Transaction, Digital, Payment, Net banking*

1. Introduction:

Cashless economy is the economy which can be defined as a situation in which the flow of cash in an economy is not done and all the transactions of the economy is done by the electronic ways. When demonetization is done then people have no money to buy some commodities, so there is Cashless system evolved. A cashless economy is an economy in which all types of transactions are carried out through digital means. It includes e-banking, debit cards, credit cards, card-swipe machines and digital wallets. We study that how many people use the cashless transaction. We also find out mostly used the mode of cashless transaction amongst the people. We check the cashless transaction is dependent or not on the gender, occupation and education also carried out proportion test to check various hypothesis.

2. Objectives:

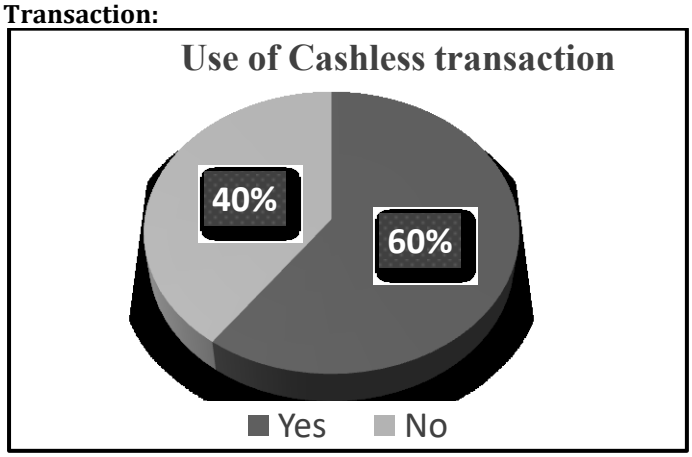
i. To find out how many people use Cashless transaction.
ii. To find out relation between education and use of Cashless transaction.
iii. To find out relation between occupation and use of Cashless transaction.
iv. To find out relation between gender and use of Cashless transaction.
v. To find out mostly use mode of Cashless transaction.
vi.

3. Data Collection:

The primary data is collected for this project. A well questionnaire is designed to collect the data from various areas of the vita city. The data is collected by using a sampling method. 200 samples were taken from various areas of vita city.

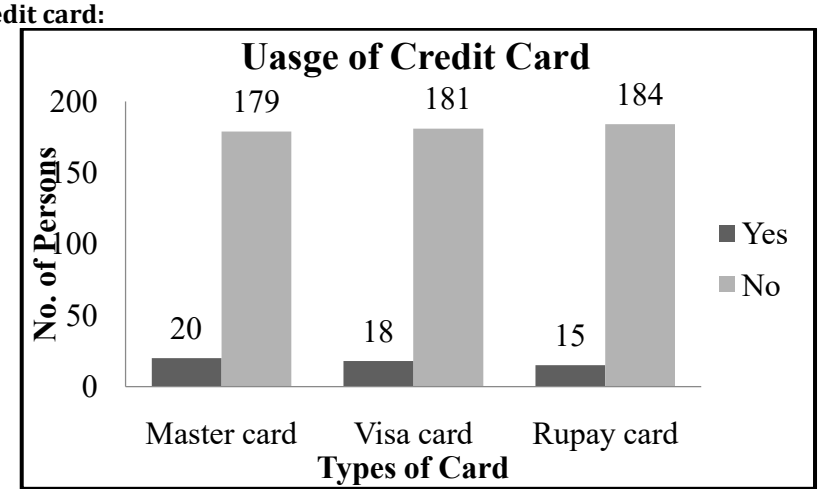
4. Statistical Data Analysis:

People using Cashless Transaction:



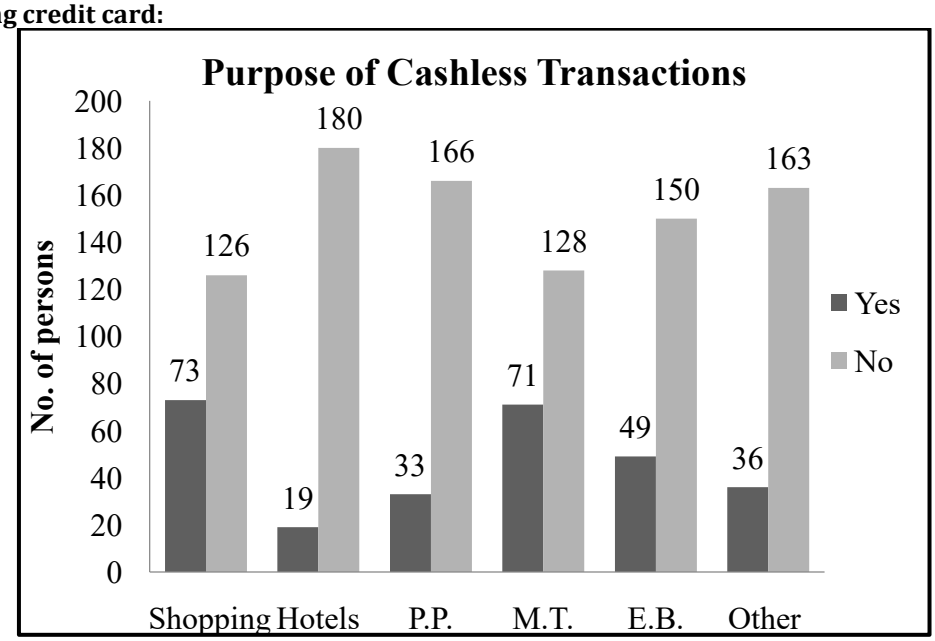
Here about 60% of the people use Cashless transaction and about 40% of the people not use Cashless transaction.

People using credit card:



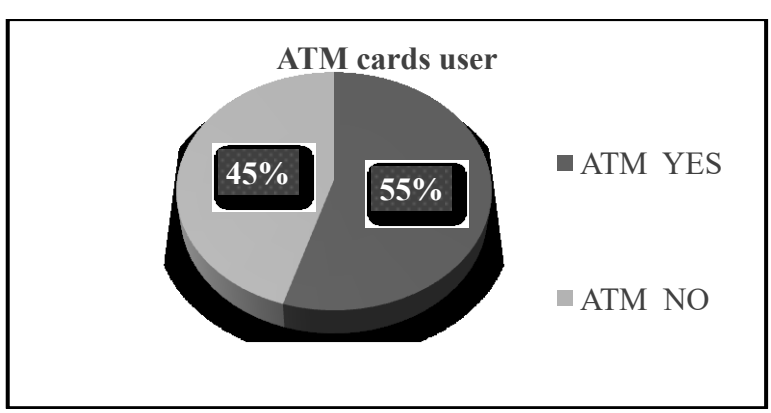
There are three types of credit cards, in which most of the people use Master card than Visa card and Rupay card.

People using credit card:



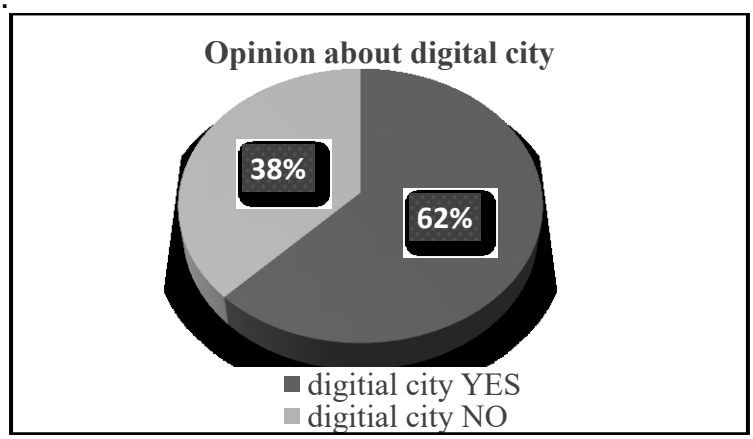
Most of the people use online transaction for online shopping, money transfer. About 19% of people use online transaction for hotels. For paying electricity bill about 49% of people use online transaction.

Use of ATM:



ATM is the type of the debit card. About 55% of the people use the ATM card. Here 45% of the people not use the ATM card.

Peoples Opinion:



Here about 62% people like to live in digital city. And 38% of the people not like to live in digitalized city.

Proportion Test:

We want to tests the following Hypothesis;

i. Proportion of ATM user and non ATM user are same.
ii. Proportion of positive opinion about security and negative opinion about security are same.
iii. Proportion of positive opinion about digital city and negative opinion about digital city are same.

Hypothesis: H_0: $P_1=P_2$ Against H_1: $P_1\neq P_2$

Under H_0 Test Statistic,

$$Z = \frac{p_1 - p_2}{\sqrt{\hat{P} * \hat{Q}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \sim N(0,1)$$

Where $\hat{P} = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2}$ $\hat{Q}$ = 1-$\hat{P}$

Sr. No.	Hypothesis	Proportion values	P value	Conclusion (at 5% l.o.s.)
1	H_0:$P_1=P_2$ Vs. H_1: $P_1\neq P_2$	P_1=0.5477 P_2=0.4522	0.0711	Accept H_0
2		p_1= 0.5125 p_2= 0.4874	0.6884	Accept H_0
3		p_1= 0.62 p_2= 0.38	7.47e-07	Reject H_0

Chi-square Test for Independence:

We want to test the independence in following hypothesis:

1. There is no dependency between gender (A) and use of Cashless transaction (B).
2. There is no dependency between occupation (A) and use of Cashless transaction (B).
3. There is no dependency between education (A) and use of Cashless transaction (B).

Under H_0, Test statistic,

$$\chi^2 = \sum_{i=1}^{r}\sum_{j=1}^{s} \frac{[(A_iB_j) - (A_iB_j)_e]^2}{(A_iB_j)_e} \sim \chi^2_{(r-1)(s-1)}$$

Sr. No.	Hypothesis	P value	Conclusion (at 5% l.o.s.)
1	Ho: A & B are independent. Vs. H1: A & B are dependent	3.204e-05	Reject H_0
2		2.252e-05	Reject H_0
3		0.01126	Reject H_0

5. Findings:

i. Mostly people use the online transaction. About 60% of the people use the online transaction.
ii. Most of the people use cashless transaction for online shopping and money transfer.
iii. Proportion of ATM user and non ATM user are same.
iv. Proportion of positive opinion about security and negative opinion about security are same.
v. Maximum number of people have desire for digital city.
vi. Gender, occupation and education are dependent on Cashless transactions.

6. References:

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A STATISTICAL ANALYSIS OF PETROLEUM PRODUCTS: CASE STUDY

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ABSTRACT: *Petroleum is most vital source of energy, providing over 50 percent of all commercial energy consumption in the world. Petroleum products are material derived from crude oil as it is processed in oil refineries. The majority of petroleum is converted to petroleum products, which includes several classes of flues. Various grades of fuel oil and gasoline are divided in to jet fuel, diesel fuel, heating oil and heavier fuel oil. This paper presents and proposes a different approach of time series analysis on prices of petroleum products, fitting of appropriate model, determine the effect of prices for different periods and finally forecast the prices of petroleum products.*

Keywords: *Petroleum products, Time series analysis, Forecasting, Regression analysis.*

1. Introduction

Petroleum is most vital source of energy, providing over 50 percent of all commercial energy consumption in the world. The sole of petroleum products began in December 1957, managed by consortium of Royal Dutch Shell & British petroleum B.P. now known as Shell petroleum development company SPDC.

Petroleum products are material derived from crude oil as it is processed in oil refineries. Unlike petrochemicals, which are collection of well-defined usually pure chemical compounds, petroleum products are complex mixtures. the majority of petroleum is converted to petroleum products, which includes several classes of fuels.

According to composition of crude oil & depending on demand of market, refineries can produce different shares of petroleum products. The largest share of oil products is used as energy carriers i.e. various grades of fuel oil and gasoline. This fuel includes or can be blend to give gasoline, jet fuel, diesel fuel, heating oil and heavier fuel oil.

2. Statistical Analysis:

1) Quartiles

2014	Petrol	Diesel	Speed petrol
Q_1	73.82	61.2275	78.1875
Q_2	76.43	63.46	82.255
Q_3	80.195	64.9125	89.29

Conclusions:

1. The 50% data of petrol is below than 76.43, there is not much difference within first and second quartiles.
2. The 50% data of diesel is below than 63.46, there is not much difference within third and second quartiles.
3. The 50% data of speed petrol is below than 82.255, there is not much difference within first and second quartiles.

2015	Petrol	Diesel	Speed Petrol
Q_1	66.095	52.415	68.69
Q_2	66.72	53.77	69.52
Q_3	69.72	55.405	72.38

Conclusions:

1. The 50% data of petrol is below than 66.72, there is not much difference within first and second quartiles.
2. The 50% data of diesel is below than 53.77, there is not much difference within first and second quartiles.
3. The 50% data of speed petrol is below than69.52, there is not much difference within first and second quartiles.

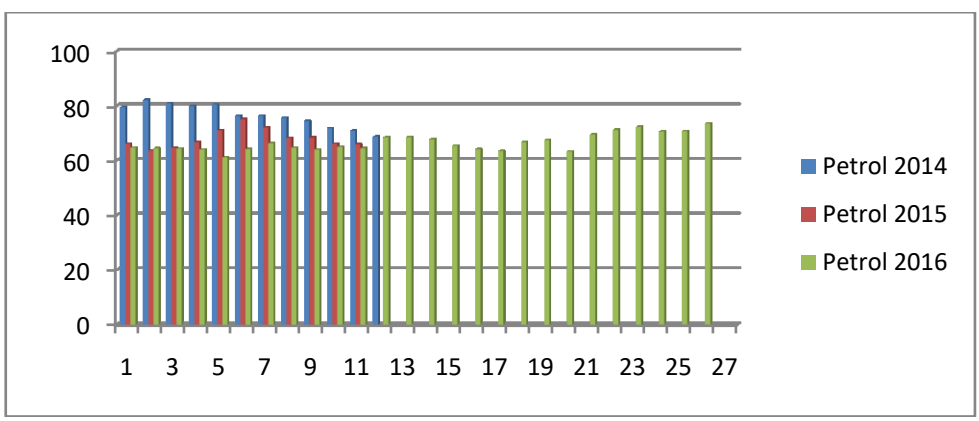
2016	PETROL	DIESEL
Q_1	64.25	53.67
Q_2	65.295	55.55
Q_3	68.5675	58.84

Conclusion:

1. The 50% data of petrol is below than 65.295, there is not much difference within first and second quartiles.
2. The 50% data of diesel is below than 55.55, there is not much difference within first and second quartiles.

2) Graphical Representation:

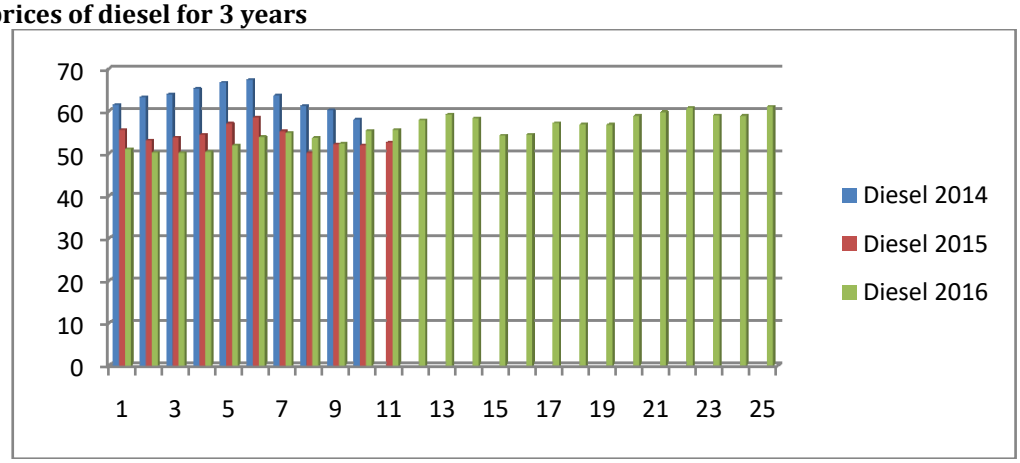
I) The prices of petrol for 3 years



Conclusion:

1. In 2014, the prices of petrol have no more variations.
2. In 2015, the prices of petrol is increases and then decreases.
3. In 2016, the prices of petrol goes on increasing.

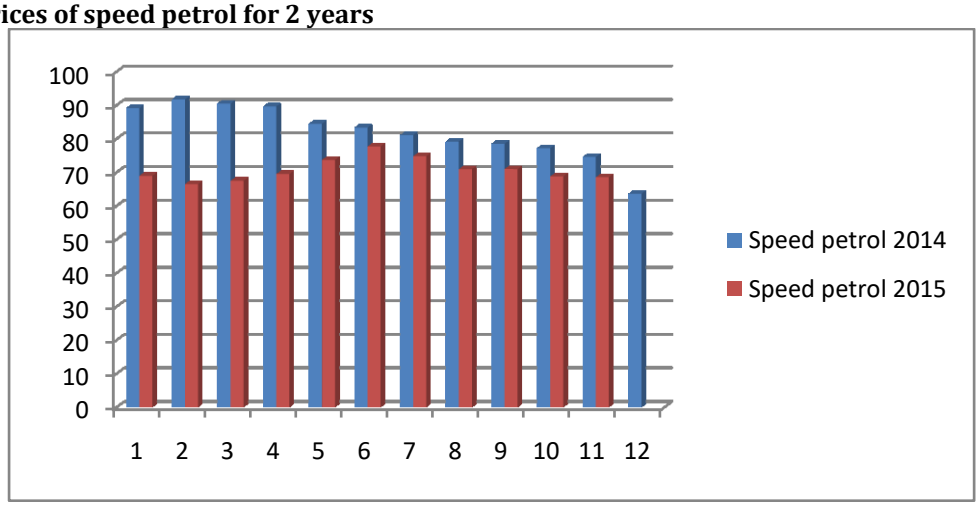
II) The prices of diesel for 3 years



Conclusion:

1. In 2014, the prices of diesel is increases and then decreases.
2. In 2015 and 2016, the prices of diesel have no more variations.

III) The prices of speed petrol for 2 years

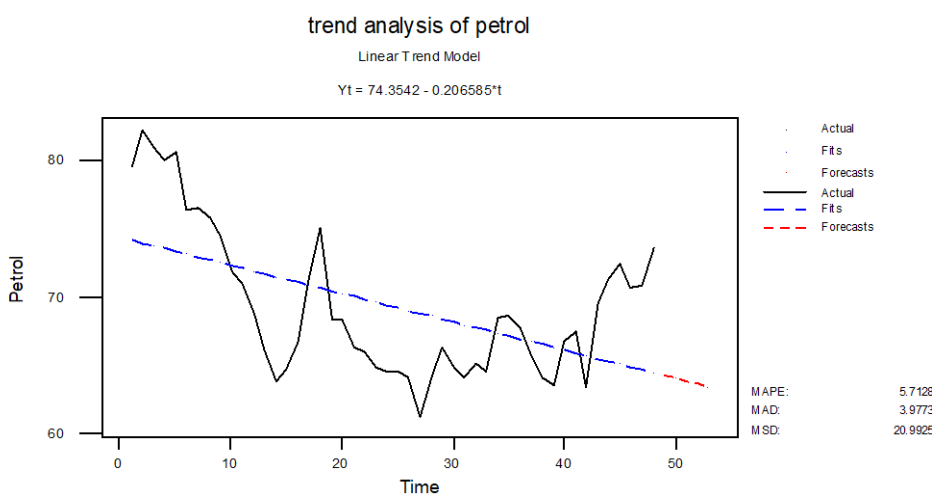


Conclusion:

1. In 2014, the prices of speed petrol goes on decreasing.
2. In 2015, the prices of speed petrol increases and then decreases.

3. Trend Analysis:

1)



Data	Petrol
Length	48.0000
N Missing	0

- Fitted Trend Equation:

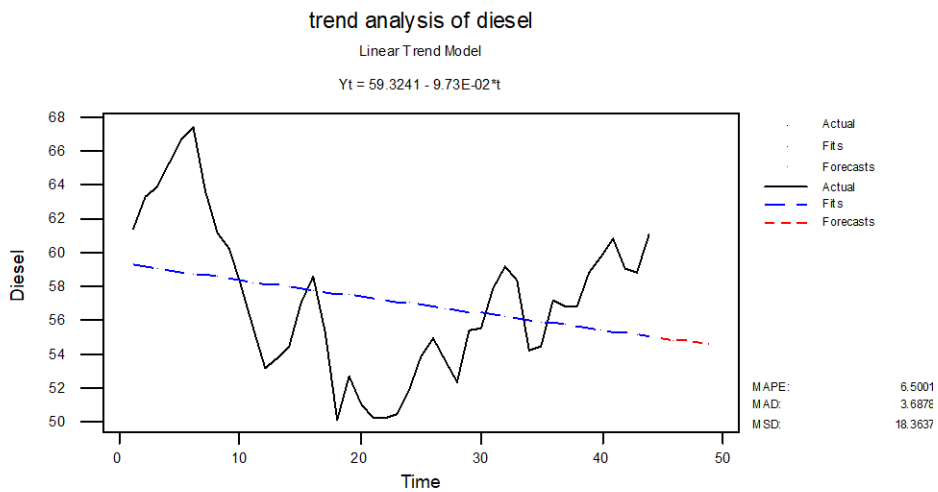
$$Yt = 74.3542 - 0.206585*t$$

- Accuracy Measures:
  MAPE: 5.71280
  MAD: 3.97733
  MSD: 20.9925
- Forecasted prices of speed petrol for next 5 period

Row	Period	Forecast
1	49	64.2316
2	50	64.0250
3	51	63.8184
4	52	63.6118
5	53	63.4052

- The trend of prices of petrol is irregular

2)



Data	Diesel
Length	44.0000
N Missing	0

- Fitted Trend Equation

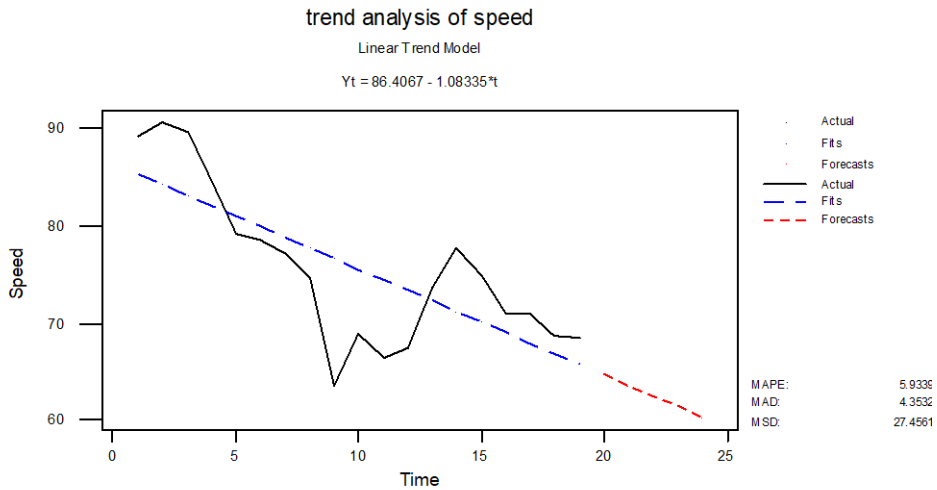
Yt = 59.3241 - 9.73E-02*t

- Accuracy Measures:
  MAPE : 6.50005
  MAD : 3.68777
  MSD : 18.3637
- Forecasted prices of speed petrol for next 5 period

Row	Period	Forecast
1	45	54.9450
2	46	54.8477
3	47	54.7504
4	48	54.6531
5	49	54.5558

- The trend prices of diesel is irregular.

3)



Data Speed Petrol
Length 19.0000
N missing 0

- Fitted Trend Equation:

Yt = 86.4067 - 1.08335*t

- Accuracy Measures:

MAPE: 5.93388
MAD: 4.35321
MSD: 27.4561

- Forecasted prices of speed petrol for next 5 period

Row	Period	Forecast
1	20	64.7396
2	21	63.6563
3	22	62.5729
4	23	61.4896
5	24	60.4062

- The trend prices of speed of petrol is irregular.

4. Conclusion:

1) In 2014,
   i. the prices of petrol have no more variations.
   ii. the prices of diesel is increases and then decreases.
   iii. the prices of speed petrol goes on decreasing.
2) In 2015,
   i. the prices of petrol is increases and then decreases.
   ii. the prices of diesel have no more variations.
   iii. the prices of speed petrol increases and then decreases.
3) In 2016,
   i. the prices of petrol is increases and then decreases.
   ii. the prices of diesel have no more variations.
4) From the trend analysis, the prices of petrol, diesel and speed petrol are irregular.

5. References:

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