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RESEARCH ARTICLE

Automatic Text Summarization of Articles in Wikipedia

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ABSTRACT

This paper proposes a summarization system for summarization of Wikipedia article. In modern years, there has been an explosion in the quantity of text data from a wide spread of sources. This large amount of text is a helpful source of information and knowledge that must be effectively summarized to extract the hidden interesting information. The main aim of this work is the reduction of a given text to a smaller number of sentences without leaving out the main ideas of the original text. Text Summarization is a powerful text mining technique for extracting the useful information that present in the document without loss of context and information. It decreases the text by withdrawing the less valuable information which encourages the user to locate the required information without delay. This work carried out based on techniques for sentence extraction and aim to cover the set of sentences that are most significant for the understanding of a given document. Text summarization work is split into four main phases. It comprise of Input text, Pre-processing, applying Text summarization algorithm and evaluation. Frequency summarizer is selected as an algorithm to generate Wikipedia articlesummary.

Keywords: Text summarization, Python, Wikipedia, Text mining, Sentence Weight

INTRODUCTION

With the enormous availability of text document on the internet, it gives more details than is required. It is extremely hard for individuals to physically summarize huge reports of text. So searching for relevant documents through an overwhelming number of documents available in the web is a very difficult task. So as to take care of

the over two issues, the automatic text summarization is especially important. Automatic text summarization has been growing in importance with the rapid growth of on-line information services. The objective of text summarization is to take a source input text and present the most significant content in a condensed structure to the necessities of the user and the task. Summarization is a hard problem of natural language processing (NLP) because, to do it properly, one has to really understand the point of a text**.** A Summary can be used in a symbolic manner as a reference to other sections of the original document, or in an informative manner to cover all relevant text details. In the two cases the most significant benefit of utilizing a summary is its less reading time.

Text summarization can be classified into extractive and abstractive summarization. An extractive summarization procedure comprises of picking vital sentences, paragraphs and so on from the source input and concatenating them into shorter type. Many extractive approaches for automated summary generation have been developed over decades that apply a range of machine learning and optimization techniques. An abstract summary [9][10] aims to build an understanding of the key concepts in a text and the n articulate those concepts in a simple, natural language. It utilizes semantic strategies to inspect and decipher the text and afterward to locate the new ideas to depict it by producing another shorter text that passes on the most significant information from the source document. The rest of the paper is formed as follows. Section 2 discusses with the related work in text summarization. Section 3 presents the overview of proposed methodology along with the algorithm. Section 4 shows the result and discussion. Section 5 is about the conclusion and future work.

Related Work

In literature most of works have been concentrated on the sentence-extraction method. This review mainly focuses on statistical method used for sentence scoring. The authors [2] proposed a clustering algorithm for sentences centered on a database. The sentences are defined as vertex and the relationship is based on the four distinct relationships such as semantic similarity, statistical similarity, discourse relationships and resolution of co-reference. In this paper [3] author has explained the over-all idea of extractive summarization and all possible extractive summarization challenges in the paper. The author also clarified the features used to produce a summary and features previously used and listed several of the extractive summary techniques to infer the difficulties and summarize the use of these techniques. According to author the importance of sentences is determined on the basis of sentence statistical and linguistic features. In [4] author has described the word level features and sentence level features. And also categorized all extractive summarization methods into unsupervised and supervised methods and have explained each method and have depicted few evaluation metrics [4].

In [5] author has explained different extractive summarization features and the summarization process structure basically comprises of two steps pre-processing and processing. The Author has described different extractive summarization methods and also describes a comparative study of different extractive summarization methods explaining each method's advantages and disadvantages. He has also clarified two MEAD and summarist summarizer methods. The authors described both extractive summarization technique and abstractive summarization technique and have described text summarizers and summarization tools for Indian languages and presented a contrast between performances of different methods in the paper A Review Paper on Text Summarization [7].

In [8] the authors, proposed an abstractive based summarization system based on semantic graph method for Tamil document summarization. The authors in [9] proposed a model to improve the quality as well as performances of the summary generated by the clustering technique by cascading it with Support-Vector- Machine (SVM). The various similarity measures are utilized in order to identify the similarity between the sentences of the document and then they are grouped in cluster on the basis of their term frequency and inverse document frequency (tf-idf) values of the words. The proposed method [10] uses sentence ranking of a topic specific document to generate

automatic summary. The method is based on the concept of extractive summary, in which the summary of a document is obtained by scoring, ranking and selecting the highest ranked sentences of the document. Experiments on these methods were conducted to compare the results.

METHODOLOGY

Automatic text summarization is a process of minimizing the size of source document by searching for important contents and expressing it in a form which is less than half of the main text. Summarization is automatic when it is generated by software or an algorithm. The major types of automated methods for summarizing text can be categorized as extractive and abstractive summarization. The automated text summarization system uses the method of extraction to produce a summary. Sentence extraction procedures are regularly used to create extraction summaries.

The basic idea for creating a summary of any document includes the following:
Step1: Text Preprocessing - removal of stop words, punctuation
Step 2: Feature Extraction - how many times each word appears in the document
Step 3: Score each sentence determined by the words it contains
Step 4: Build summary by joining each sentence over a specific score limit

Term Frequency

The term frequency is very significant component. TF (term frequency) be a symbol of what number of time the term repeatedly occurrence up in the document (usually a compression function such as square root or logarithm is applied) to determine the term frequency. The term realizing sentence endpoint in an input document relies upon on punctuation for instance, (. (, “, [, {, etc.) and spliting into sentences. These sentences are just tokens. Term-frequency is typically 0 or 1 for sentences-since normally the similar content-word does not appear to be generally in a given sentence.

Weighted Frequency of Occurrence

To find the weighted frequency of occurrences it does not contain punctuation, digits or other extraordinary characters. It can locate the weighted frequency of each word by partitioning its frequency by the frequency of the most taking place word.

Weighted Frequency in Sentences

The weighted frequency is finding its sum instead of the corresponding terms in place of the corresponding words in the original sentences and finding their sum. It is important to mention that weighted frequency for the words removed during preprocessing (stop words, punctuation, digits etc.) will be zero.

Sentence Score

After converting the input text document into intermediate representation, score (or weight) is assigned to each sentence of the given document to identify the important sentences. The weight of each sentence is determined by examining the different parameters used in the method.

Sort Sentences in Descending Order

To sort the sentences in inverse order of their sum. The sentences with highest frequencies summarize the text. The sentence with the highest sum of weighted frequencies. The sentences are organized in descending order based on the acquired sentence score. Out of these sentences top N sentences are chosen based on compression rate provided by the user.

RESULT AND DISCUSSION

The information that is to be embedded plays a vital role. In order to get the meaningful output, the input should be acceptable and understandable. The dataset used here is a text document of l Wikipedia article in the domain of Artificial intelligence. The size of the dataset is 176 KB. The experimental evaluation of the text summarization algorithms are executed on Wikipedia article and corresponding top-N (7sentences) summaries generated for each of documents. The proposed automatic text summarization based on single document system is implemented in the PYTHON program and the text summarization process is experimented with the document is collected from WIKIPEDIA Article. There were a number of different Python packages and modules used for different areas of functionality across this work. The Figure 3 shows the input text taken for experimental purpose. Figure 4 shows the Word frequency for the words present in the given input document. Figure 5 shows the Sentence Score for the words present in the given input document. Figure 6 shows the Final Summary generated from the input file based on the sentence score. For examination, the summary is produced for various compression rates and the created summary is assessed utilizing the measures precision, recall and F-measure denoted in Table 1. The execution of the algorithm is estimated with online tool summarize bot. The similarity graph of the precision, recall and F-measure is displayed with different charts were mentioned in Figure 7. By analyzing with C=30%, the result of recall and F-measure shows greater performance.

CONCLUSION AND FUTURE SCOPE

Because of the immense measure of text information accessible on Web which can't be examined by people, an administration arranged methodology can be helpful for recovering significant information from text documents. Summarization is basically valuable since it gathers information for simpler utilization and analysis. This work is developed a text summarizer that is capable of producing a summary from Wikipedia article as per the number of lines requested by the user. The proposed technique is basically an extraction based methodology. The extractive approach involves choosing the top N most sentences that best spread the entire information uttered by the original source content. This is the most well known methodology, particularly in light of the fact that it's simpler than the abstractive methodology. The proposed system is only for text document but in future it is plan to develop text summarization model for all types of document including graph, images, picture and video moreover to the text document. Performance evaluation of algorithm will be done with human summary instead of online tool summary.

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Table 1: Result of Frequency summarizer compare with online Tool summary

	10%	20%	30%
Precision	0.11	0.18	0.28
Recall	0.13	0.20	0.39
F-Score	0.11	0.18	0.32

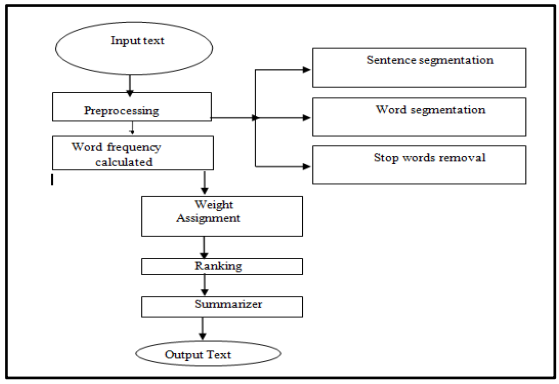

Fig. 1: Frequency summarizer

Step 1: Convert Paragraphs to Sentences Step 2: Text Preprocessing

Step 3: Tokenizing the sentences

Step 4: Find Weighted Frequency of occurrence

Step 5: Replace Words by Weighted Frequency in original sentences

Step 6: Sort Sentences in Descending order of sum

Step 7: Generate summary

Fig. 2: Frequency summarizer Algorithm

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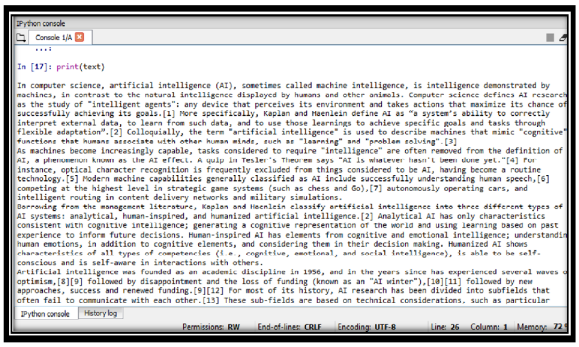

Fig. 3: Input file taken from the Wikipedia

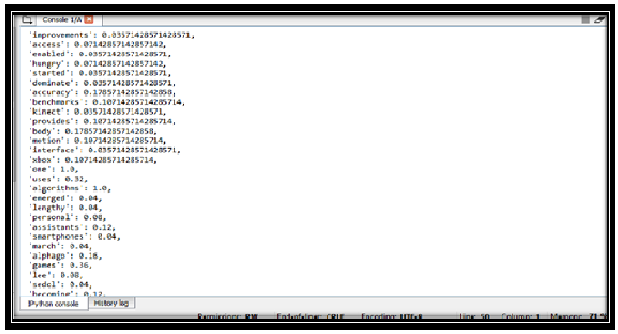

Fig.4: Word Frequency calculation

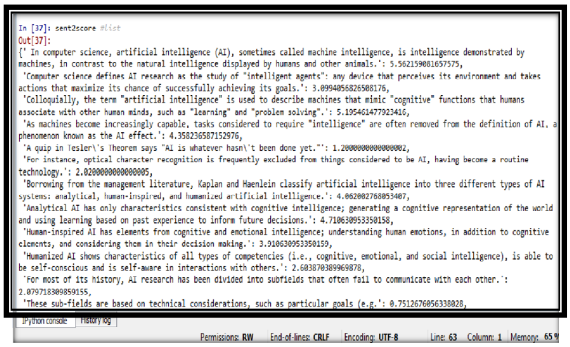

Fig. 5: Sentence Score for the input document

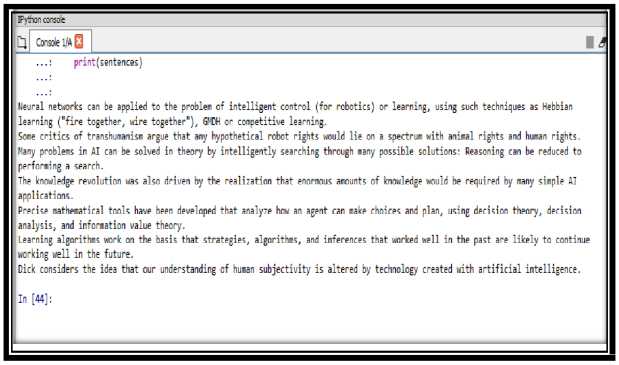

Fig. 6: Summary output for the Article in Wikipedia

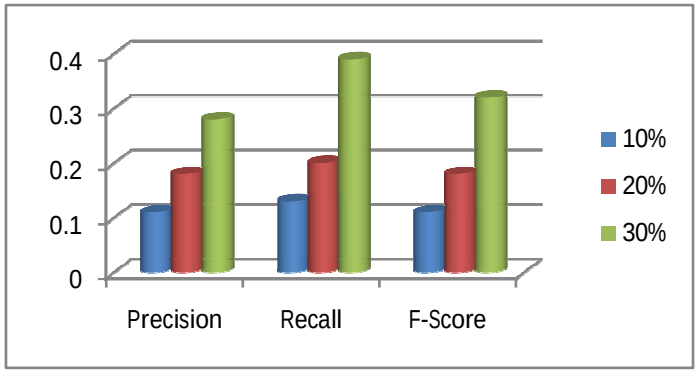

Fig. 7: Similarity Chart for the Precision, Recall and F-Score

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REVIEW ARTICLE

A Review on the Progress of Renewable Energy Investments in the Gulf Cooperation Council Countries Concerning Saudi Arabia

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ABSTRACT

Saudi Arabia is the world's top oil producer and exporter and one of the country's that generate power directly from crude oil. Burning crude oil produced a large amount of carbon dioxide in the atmosphere and is negatively affecting the ecosystem. In this regard, renewable energy can play an essential role in generating electricity without a need to fuel and to reduce the environmental hazard. This study aims to provide an overview of the progress of renewable energy concern to the investment decision in Saudi Arabia. It uses different literature-based analyses to identify the critical reasons for investments, also what drivers and barriers are presently affecting the renewable energy investment decision in the country. The findings highlight the enormous geographical potential of Saudi Arabia. It among the best countries known for solar and wind energy also has a large regional market, investor centric economy, and knowledge-based economy are the other main reasons that support investors to invest in Saudi Arabia. However, some obstacles: inefficient bureaucracy, a combination of fuel/electricity subsidies, and a lack of efficient support system sometimes prevent investments in renewable projects. The study results support that there is a need for strong government support in the long run, as a way to diversify economies and prepare future without fossil. More specifically, government role, education status, and public and private investor's participation appear crucial for investment decision and particularly for the overall economic development of the KSA.

Keywords: Renewable Energy, Crude Oil, geographical potential, KSA

INTRODUCTION

Saudi Arabia is the largest country in the Arabian Peninsula, with a total area of 2,149,690 squarekilometers and a population of27,019,731 million (2006 census). Saudi Arabia is located in the southwest corner of Asia and at the crossroads of Europe, Asia, and Africa. It is surrounded by the Red Sea in the west, by Yemen and Oman in the south, the Arabian Gulf and the United Arab Emirates and Qatar in the east, and Jordan, Iraq, and Kuwait in the north ("Geography of Kingdom of Saudi Arabia," 2019). GCC country, Saudi Arabia's energy sector, primarily includes petroleum, natural gas production, consumption, exports, and electricity production. It is one of the world's largest producer of crude oil, and its economic growth is tied closely to oil prices. However, like many industrialized nations, KSA also has initiated long-term plans to replace its current energy sources with renewable and sustainable energy sources. Kingdom of Saudi Arabia (KSA) in 2016 announced its Vision 2030 strategic plan incorporating significant changes to the economic structure of the country, including an intention to deploy 9.5 GW of renewable energy to reduce the penetration of oil in the electricity generation system(Fattouh & Sen, 2016).

Under Saudi Vision 2030 and the National Transformation Program, the National Renewable Energy Program (NREP) has launched in the year 2016. NREP is a long term, multifaceted renewable energy program designed to balance the domestic power mix while working towards carbon reduction commitments. As per the report of Saudi Invest (2018), KSA has to adopt new long-term energy strategies to reduce the share of fossil fuels in primary energy consumption. The program is managed and executed by the Ministry of Energy, Industry, and Mineral Resources (MEIM), directly supporting Saudi Arabia's Vision 2030. The Program will be phased and rolled out systematically and transparently to ensure that the Kingdom benefits from the cost-competitive nature of renewable energy. The Program's main objective is to substantially increase the share of renewable energy in the total energy mix, targeting the generation of 27.3 gigawatts (GW) of renewable energy by 2024 and 58.7 GW by 2030 (Fattouh & Sen, 2016; REPDO, 2017)(Salam & Khan, 2018)(Saudi Invest, 2018).

In this paper, we investigate the current status of renewable energy in Saudi Arabia, also whether an investment opportunity for renewable energy in Saudi Arabia is present, what current obstacles stand in the way of renewable future. The remainder of this paper is structured as follows. Based on Vision 2020, the next section provides a review of the key reasons to invest in renewable in Saudi Arabia based on vision 2020. Section 3 and 4 provide a literature review with the main divers and barriers to renewable energy investment in Saudi Arabia. Finally, the conclusion regarding what it would take for vast scale expansion to happen in the future

Key Reasons to Invest in Renewable Energy in the Kingdom of Saudi Arabia: Unlimited Natural Potential for Renewable Energy Production

GCC countries have both abundant fossil fuel resources and vast renewable energy potential. It is visible that GCC countries have immense potential for wind, solar, and geothermal energy generation. Among all renewable energy sources, wind and solar energy are the most promising renewable energy resources (Gastli & Armendáriz, 2013; Lilliestam & Patt, 2015).Among all GCC countries, Saudi Arabia has immense potential in solar and wind energy production, ranking as the sixth and 13th country, respectively (Saudi Invest, 2018).
Saudi Arabia lies in the middle of the "sunbelt" and has an average of 8.9 hr/day **sunshine**, and horizontal solar radiation of 5,600 Wh/m^2. Solar irradiation in the country is well above average irradiation of high potential solar areas globally 250 w/m^2vs. 100-200 w/m^2. Onshore wind speed in Saudi Arabia is around 6.0m/s and 8.0 m/s, and the vast areas onshore wind speed is well above a standard economic viability speed. KSA's most of the provinces have a high potential of solar mainly Tabuk and Asir. Unlike solar, the potential of wind energy lies along the northeast, central regions, and mountains in the western region.

As per the Report of Saudi Invest (2018), KSA ranked as the 6th country with the highest potential for producing solar energy and 13th with the highest potential for the production of onshore wind energy. Among the top 10 countries having solar potential, KSA has solar potential approximately 33.9 K Petajoule/Year. KSA listed as the 13th country with the highest potential for the production of onshore wind with 0.5 K Petajoule/Year (Saudi Invest, 2018)

The Support and Commitment of the Government to the Renewable Energy Sector

The quality of life and safeness of the present and future generations strongly connected with the availability of energy sources and the sustainability of the energy infrastructure (Stambouli, 2011). After the emission of the Kyoto protocol, many counties, including GCC countries, have realized that the abundant use of fossil fuel harms the environment as well as on health (Gastli & Armendáriz, 2013). Saudi Arabia is a leading oil producer stillkeenly interested in taking an active part in the development of renewable energy sources and rational use of energy. During the last few years, political support for renewable energies has been growing continuously both at the national and international levels. In this context, Saudi Arabia participated in many international conventions to protect the global environment and reduce Greenhouse emissions (Doukas, Patlitzianas, Kagiannas, & Psarras, 2006; Stambouli, 2011). In addition to this natural endowment, the government's goal to achieve environmental sustainability has led to the emergence of renewable energy as the main objective in Vision 2030 (Fattouh & Sen, 2016).

A Sizeable Regional Market in GCC

KSA, with a population of 32 million and the largest economy in the Arab World with a GDP of USD 683.7 billion located proximity to the other GCC countries, thus has the logistical advantage: lower transportation cost, shorter delivery time over other countries ex. European and Asian countries(Saudi Invest, 2018). It is the only G-20-member country in the region. Saudi Arabia has almost 16 percent of the world's proven petroleum reserves, plays a principal role in OPEC, is one of the world's largest producers and exporters of crude oil, and is a large-scale oil refiner and producer of natural gas (The International Trade Administration (ITA), 2017). In recent times to KSA has set many favorable trade regulations to support the export of industrial products and equipment. It is one of the member country GAFTA, i.e., trade agreements with other Arab countries. Under the GFTA, goods produced in any GAFTA member countries shall be exempted from duty in any other member country.

Knowledge-Based Market

According to Powell (2011), through the centuries, knowledge has been used to fascinate and liberate. In contrast, a knowledge market is a mechanism for distributing knowledge resources and has always commercialized(Jeniffer-Kite, 2011). Hence, knowledge has become a vital economic resource, especially as the basis of economic growth. However, knowledge also is a force in other social institutions of modern society, including, of course, in governance or the world of work (Macias Vazquez & Alonso Gonzalez, 2016).

In recent years the world economy is witnessing a fundamental structural change driven by globalization and ICT, leading to a new economic system characterizing by increasing importance of knowledge. Hence, knowledge creation, accumulation, and acceleration are intensified the pace of scientific and technological progress and have been at the heart of economic growth literature. Saudi Arabia is facing an unprecedented pace of change across all aspects, including social, cultural, economic, and even regulatory. Many critical projects in the Kingdom demonstrate an actual shift towards a knowledge-based economy, with a significant focus on technology (Young, 2018). According to Dr. Abdul Aziz Alsebail, the secretary journal of the King Faisal International Prize, a knowledge-based economy is pivotal in achieving sustainable development(Nour, 2014; Rashid, 2018). Saudi Arabia has achieved significant improvement, rapid and fastest progress since 2000 not only by the regional standard but also by international standards. The progress appears from improvement in terms of KI, KEI, ICT pillar, education pillar,

economic incentive and institutional regime pillar, innovation efficiency index, knowledge creation index, knowledge impact index, knowledge diffusion index, and technological infrastructure, (Nour, 2014).

Investor-Centric Ecosystem

During 2017, the Saudi Arabian government took several positive steps to improve the investment climate in the Kingdom. The government moved forward with plans to privatize many state-owned entities across a range of sectors, including transportation, education, energy, and healthcare, albeit at a gradual pace (The International Trade Administration (ITA), 2017). The projected growth in both renewable energies, together with the ongoing privatization, localization, and energy efficiency efforts, leads to attractive investment opportunities for private sector investors in the Energy sector. Climate change and meeting future energy demand is the primary reason for investment in renewable energy, and investment in renewable technology can provide great potential as an alternative source to supply electricity to the growing communities of the modern world(Gastli & Armendáriz, 2013). Development of NREP with the help of the Ministry of energy and Renewable energy project development office, KSA established transparent governance set for renewable sector investments. NREP helps in reassuring investors to enhance credibility and provide transparency and enhance assurance to the private sector, and raising confidence in the market (Fattouh & Sen, 2016).

Drivers for Renewable Energy

One of the most important aspects of any country is to preserve the natural environment and balance the exploitation of resources for the requirement of life and future generations as well as economic development. It is evident by studies that the world is warming. In recent times the emission of carbon comes at the rising level due to brisk economic expansion and population growth, the demand for energy and electricity, and raising global concern over climate change and global warming (Bhutto, Bazmi, Zahedi, & Klemeš, 2014; Radhi, 2009). this context, climate change, and global warming have become severe and growing concerns in recent years. (Bhutto et al., 2014; Moriarty & Honnery, 2012; Radhi, 2009).

Some scientific studies believe these recent changes in the environment are due to natural variability, while other groups of studies indicate to human activities. These other groups of studies believe human activities as the leading cause of increasing greenhouse gases and a key driver of climate change. Michael (2006) supports these believes as they highlighted the significant devastating impact of human activities on the environment with the capacity of societies to survive. (Jefferson, 2006; Radhi, 2009). The increase of energy consumption as a result of human activities in the GCC region is, to a certain extent, an inevitable outcome of social and economic development (Asif, 2009; Doukas et al., 2006). The social and economic development challenges of the country, the demand for energy, and particularly for electricity, are proliferating.

Since fossil fuels continuously become more expensive and advent depletion of reserves, another side, depletion results into greenhouse gas emission their use as an energy source harms the environment, the use of renewable energy will provide for most of the energy in the future as well as sustainable development to several energy-related environmental problems (Moriarty & Honnery, 2012; Rourke, Boyle, & Reynolds, 2009). The government recently heralded a future without oil, and renewable energy resources can be used as one of the methods to fulfill the increasing demand and to secure the economy. It is the appropriate time to invest in developing capabilities in renewal energy in order to secure the future (Al-Saleh, 2009; Gastli & Armendáriz, 2013). The use and development of renewable energy on the whole fueled by a range of environment, energy security and or economic consideration and could significantly improve environmental protection and continuing oil supplies in conditions of stability (Al-Saleh, 2009; Lilliestam & Patt, 2015)

Barriers for Renewable Energy

Though Middle East countries have vast renewable energy sources and sizable geographical potential, yet the development of renewable energy in these countries is shallow. Johan and Anthony (2015) investigated in a literature meta-analysis and survey and noted that there is a long-term need for renewable, to diversify the economy, and prepare for the post-fossil era (Bhutto et al., 2014; Lilliestam & Patt, 2015). However, what barriers and risks presently deter investments, and possible policy solutions could use yet not determined empirically. Concerning the obstacles of renewable energy, Johan and Anthony (2015) found the main obstacles deter investments: inefficient bureaucracy, the combination of fossil fuels/electricity subsidies, and absence of support system. Konstantinos and John (2006) noted that the renewable energy market has always stifled by the combination of constraints. The absence of a relatively legal and policy framework, the high capital costs, and lack of marketable skills are some of the critical constraints and put the renewable at an economic, regulatory, or institutional disadvantage(Patlitzianas, Doukas, & Psarras, 2006). Jefferson (2006) addressees lack of political leadership and courage are the reasons forlow energy efficiency and conservation measures over the past years(Jefferson, 2006). In summary, there are many studies on renewable energy based on Middle East countries. Most of these studies examined the sources, technologies, potential of renewable energy in the Middle East countries, overlooking the constraints that deterring investments of renewable energy. Many of these studies have based on literature studies and empirical consideration yet not available. This study attempts to examine the constraints that may affect the investment decisions of renewable energy.

DISCUSSION

The paper discusses the progress of renewable investment in Saudi Arabia. This research study discloses the current prospects of renewable energy in GCC countries, including KSA and the willingness of investment in Saudi Arabia. The contribution of this study is the revealed information on critical reasons for investment in renewable energy in Saudi Arabia and how the country is rated and ranked on the global level; KSA's willingness perspectives to adopt renewable energy. Results also indicate that the main factor influencing the investment decision of renewable energy is the natural endowment position as KSA ranked sixth and 13th for solar and wind potential, and revealed that the most significant energy potential investment is solar and wind.

Further study opens up the main drivers or reasons to adopt renewable energy: the emission of carbon, brisk economic expansion, population growth, the demand for energy and electricity, and global warming are essential reasons behind renewal energy. Moreover, the most important aspects of any country to preserve the natural environment and balance the exploitation of resources for the requirement of life and future generations, as well as economic development, is the main driver for renewable. The study supports there is a need for strong government support in the long run, as a way to diversify economies and prepare future without fossil fuel. In the short-term, however, investments in renewable are deterred one side by fossil fuel subsidies, high cost of investment, support system, on the other side, make the decision difficult to invest in renewable in the GCC, including KSA profitably.

Removing subsidies from fuel energy and increasing the level of awareness of renewable energy technology can be an excellent solution and enable renewable energy investments. Political, financial, social, and educational aspects should establish to allow the effective deployment of renewable projects. (Demirbas, Kabli, Alamoudi, Ahmad, & Basahel, 2017).Furthermore, findings revealed that the government role, education status, and public and private investor's participation could enable, and it appears particularly beneficial for the overall economic development of the KSA.

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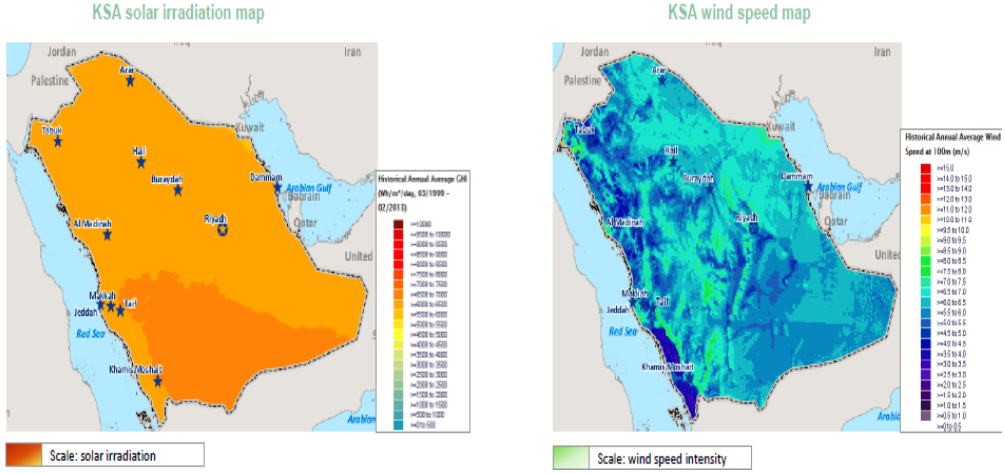

Figure 1: Source: Invest Saudi (2018) Report

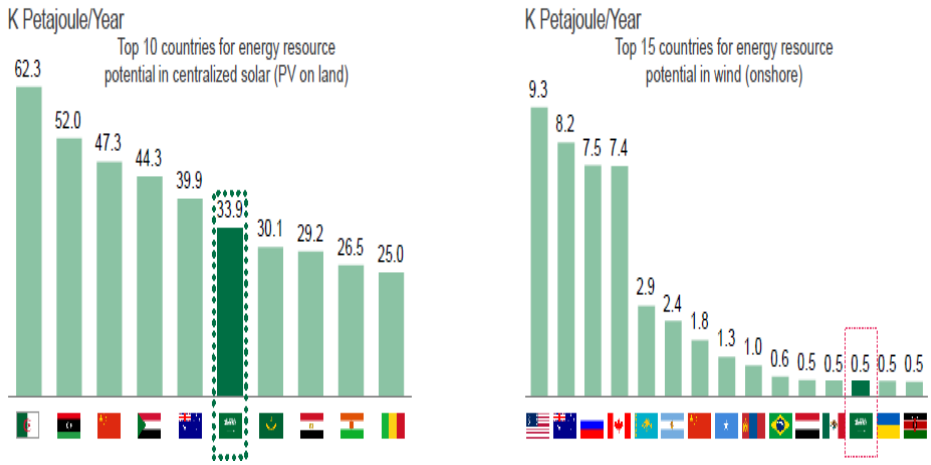

Figure 2: Source: Invest Saudi (2018) Report

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RESEARCH ARTICLE

Conversion of Pulse Waste of Pigeon Pea-(*Cajanus cajan*) into Organic Fertilizer by using *Eisenia foetida*

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ABSTRACT

Production of organic wastes from various sources due to human activity is recycled through bioconversion process for sustainable agriculture, eco-friendly environment and converted to enriched nutrient supplement for soil and plant. Huge amount of retained cooked product discharge from hotels, hostels, canteens, industries, and from various organized events etc. Out of these cooked pulse is greatly effective as it has high nutritive value for its protein aceous properties and disposal of this also impact in economic value. The utility of pulses should be economical and the waste has an option for bioconversion by using the technique vermicomposting to produce organic manure for plant growth. It is an increasing awareness about organic farming in view of the energy shortage, food safety and environmental concerns arising out of conventional farming. Organic farming helps harness of soil organism to process to animals and plant residues and to produce slow release of nutrients as needed by the crops. Earthworms and microbes are the main biological agents or biological indicators of vermicomposting for soil fertility. The present investigation deals with study of vermicomposting with respect to various proportion of waste of cooked pigeon pea (*Cajanus cajan*) or dal such as 20%,40%,60%,80% and 100% and its effects on earthworm activity. The growth and yield performance of earthworm *Eisenia foetida* which was used during vermicomposting are estimated through different physico-chemical parameters.

Keywords: Earthworm, organic waste, waste pulse, vermicompost, *Eisenia foetida*

INTRODUCTION

Tons of industrial wastes, municipal solid wastes, agricultural waste, cattle manure and poultry manure were generated from various sources and dumpedonto the crust of earth causes various environmental pollution. Environment degradation is a major threat confronting the world, and use of chemical fertilizers contribute largely to the deterioration of the environment which loss the soil fertility and adversely impacts agricultural productivity. The bioconversion option like vermicomposting, incorporation of earthworms for recycling organic wastes offers a most viable rural appropriate technology because the earthworm consumes almost 100% of their weight as wet organic wastes. Earthworms are called friend of farmeralso known as biological indicators of soil fertility for millions of years before the green revolution. The silent animals have been performing a marvelous function of ploughing and fertilizing soils. Earthworms prepare the ground in an excellent manner for the growth of fibrous rooted plants and for seedlings of all kinds (Darwin, 1981). Earthworms are the most important among the soil organism which are contributes to soil fertility for their activity in soils that alter soil porosity, increase in soil air volume from 8% to 30%. And it always promotes plant growth according to (Wollny,1890).

Both microorganisms and earthworms are important biological agents helping nature to maintain nutrient flow from one system to another. The present study conducts with a simple biotechnological process of vermicomposting which could provide a solution to tackle the problem of safe disposal of waste as well as the most needed plant nutrients for sustainable productivity (Wani, 2002). Vermicomposting is a simple biotechnological process of composting in which certain species of earthworms are used to enhance the process of waste conversion to better end product. Vermicomposting differs from composting in several ways. It is a mesophilic process that utilizes microorganisms and earthworms that are active at moist organic material. The vermicomposting process is faster than composting because the material passes through the earthworm gut. Earthworms are capable of transforming garbage into useful source of nutrients and organic matter. Vermicompost increase the plant growth and development as well as crop quality significantly, Hopkins (1995)

Vermicomposting is an ecofriendly technology and also a waste management thatclean the environment and provide remunerative organic manure. Vermicompostis 100% organic safe non-toxic and odour free helps plant to grow faster and stronger. Earthworm can have a significant effect on soil fertility parameters, because of their ability to modify their environment (Hauster et al 1994). The worms feed on organic material, break it down and then excrete worm casting or vermicast or vermicompost. The castings are in the form of tiny pellets which are coated with a gel. This crumb like structure helps improves soil drainage and aeration. The studies carried out under field conditions indicated that the castings of earthworms contained 2-3 times more available potassium than the surrounding soil (Basker et. al., 1993). Ellioet. al. (1990) found that earthworm castings generally have a higher ammonium concentration and water – holding capacity than bulk soil samples and they constitute sites of high denitrification potential (Ellio et. al. 1990). The organic matter also undergoes chemical changes in the process. The casting are much higher in bacteria, organic material and available nitrogen, calcium, magnesium, phosphorous and potassium and soil itself. The vermicomposting acts like a buffer for plants where soil pH levels are too high or low making soil nutrients available again to the plant. Vermicompost enhances the plant growth in field soils and green house media has been attributed to a variety of factors including physicochemical properties (Chan and Griffiths,1998; Edwards and Burrows,1988; Wilson and Carlile,1989; Mba1998; Buckerfield and Webster ,1998). Plant growth increased in potting media enhanced with vermicompost derived from animal manures (Edward and Neuhauser 1988). *Eisenia foetida* are known as the best species used for vermicomposting and vermiculture *are* otherwise known as various common name such as red worm, trout worm, brandling warm, tiger worm etc. These are adapted to decaying organic material ,thrive in rotting vegetation, compost and manure. They are epigean rarely found that move in and out to gripe nearby surface. They are also known as biological indicator of soil fertility have been performing a marvelous function of ploughing and fertilizing soils

The present investigation deals with recycling of waste pulse *Cajanus cajan* (pigeon pea) that pulses play a vital role in providing balanced protein component in the diet of the people in developing countries. Pigeon pea is the one of the most important pulse crop has grown at 5.4 million hectares in India. Pigeon pea is an important legume crop of rain fed agriculture in the semiarid tropics. Nutrition a list believes that even non-vegetarians stand to benefits by including in their diet, a reasonable quantity of pulses substitute for protein. Similarly with production most percent released as waste after cooking and consuming from various sources and the study conducted by using earthworm *Eisenia foetida* for vermicomposting of waste pulses.

MATERIALS AND METHODS

The present investigation was carried out on vermicomposting approximately about 65-75 days from January to March in the campus of CUTM located at Jatni, Bhubaneswar, Odisha, India.

Collection of Materials

The required material such as soils, cow dung was collected from garden area of CUTM, India. The cooked pulse waste (pigeon pea) was used for vermicomposting, collected from the canteen of hostels, CUTM campus, India. The cow dung free from urine was collected in a large sized rectangular plastic container from the cattle shed of the campus. Rectangular plastic pot 6 in number with 30 cm in length, 22cm in breadth and 18cm in height were collected from the market for vermiculture.

Collection of Earthworm

The exotic healthy species *Eisenia foetida* was used for the purpose of vermicomposting were brought from the vermiculture unit of the CUTM campus.

Systematic position
Kingdom- Animalia
Phylum- Annelida
Class – Clitellata
Order – Haplotaxida
Family – Lumbricidae
Genus – Esenia
Species – *E. fetida*

Preparation of Materials

The collected cow dung and soil were exposed in bright sun light for air drying and observation for drying was monitored everyday in a regular basis. The large pieces of cow dung and soil were chopped into small pieces and was allowed for further dry. The required cow dung and soil were completely dried and crushed into smaller particles .The dried materials were sieved initially through a sieve having diameter 2.36 mm. and after that the materials were brought into weighing site for the accurate measurement of the materials into different proportion.

Preparation of Vermicomposting

Experimental Setup

The experimental was designed with the following proportion by taking 6 rectangular pots.

C = Control (soil + cow dung) (1:1)
P1 = Soil + cow dung + w.pl (4:1)
P2 = Soil + cow dung + w.pl (3:2)
P3 = Soil + cow dung + w.pl (2:3)

P4 = Soil + cow dung + w.pl (1:4)
E = Experiment (100%-)

w.pl-waste of pulses. The various proportion of mixture samples were taken in different pots, labeled and kept in shaded area. Sprinkling of water was done daily upto 10 – 15 days to maintain moisture content.

Introduction of Earthworm

After slight decomposition of the composting materials i.e. after 15days about 3-4 earthworms of exotic species *E. foetida*, having length between 7-12cm are introduced into composting.

Culture of Earthworm

The earthworm were allotted into all different pots and cow dung slurry were administrated to the pots for maintain their nutrition for earthworm. Water was allowed to sprinkle in regular interval to all proportion for maintaining moisture content and the room temperature is also maintaining as same as atmospheric temperature.

Harvesting of Earthworm

In order to facilitate the separation of worms from vermicomposting, the moisture content in the compost was brought down before 4-5 days of harvesting that ensure drying of compost and migration of worms into vermiculturing pot. The remaining worm can be removed by hand. The mature compost that removed out is vermicompost from the pot, dried and packed.

RESULT AND DISCUSSION

In the present study various proportions of cooked waste pulses (pigeon peas) was experimented by using vermicomposting for production of enriched organic manure. The pH, moisture content, electroconductivity and temperature are the physico-chemical parameters easily controlled and indicated the progress of vermicomposting. The pH, electro-conductivity and moisture content were studied through graphical analysis presented in Table 2. and Figs. (1.,2,3&4) respectively. Each proportion from the present work was found varies with their physio–chemical variables such as elecrto-conductivity,pH and moisture content. The output in both Set-1 and Set-2 as was shown with positive output compared to other pots in respect to all physico-chemical parameters. Rapid consumption of organic pulse wastes by earthworm crushed into finer particle through gizzard the muscular grinding organ and released dark black granular vermicast (Lakshmi Prabha et al 2014). The nutrition for earthworms were obtained from microorganism during vermicomposting that grow upon the wastes. During pre-composting the microbes were produced and undergoes a process of fermentation which converted the mixtures into acidic medium then later reached to slightly acidic (Jadhav et al, 2013).The pH was maintained within 6.2 to 7.7 i.e. in vermicomposting pots from control to experriment in present experiment.The pH in all pots were slightly acidic except Set-3 shown alkalinity while the experimental was acidic (Table-2 & Fig.1).

It might have caused by the microbial nitrification process of nitrifying bacteria present in wastes to release volatilization of ammonical nitrogen and H+.(Eklind and Kirchmann ,2000, Singh,et al 2005).Earthworm are aerators that it emits sufficient oxygen to oxidize foul smell producing, compounds like H_2S, mercaptans, skatol, etc. to make odourless vermicompost during the process of compost formation because of the oxygen rich hemoglobin circulation through the skin of the earthworms (Nagavallemma et al , 2000)The moisture content was maintainted by sprinkling water into all pots at 65%-75% ,whereas it was more in control (Table 2. & Fig.-3). The temperature was maintained below 35^0C to avoid overheating of the wastes because earthworms to this range of temperatures even for short periods cannot survive (Taiwo and Oso, 2004). The temperature in Set-4 and experimental of present study

was shown nearer to 35^{0}C causes death of earthworm(Table2. & Fig.4).From preliminary stage of vermicomposting to till completion the earthworms *E. foetida* survived in almost all pots and population growth observed except Set-4 where it was rare and nil at experimental (Table 3. & Fig.-5,Table 4. & Fig.6).The EC was increased in initial stage due to release of different mineral salts available in mixture for the process of precomposting (Table2. & Fig.2). The available salts were later converted into insoluble salts which may be the reason for the reduction of EC as the vermicomposting process further progressed (Nisha Jain,2016). From this present study it was observed that the growth of earthworm was suitable with respect to all physico-chemical paramters available such as Set-1,Set-2 in maximum rate,Set-3 was showing with moderate value and Set-4,Experimntal acheived with minimum range in respect to standard proportion taken as control. The survival and growth of earthworm *E. foetida* was led to faster production of vermicompost in Set-1and Set-2 while less or no vermicompost was produced in other proportions of pulse waste. The end product vermicompost was obtained with Dark black granular structure, odourless and rich in soil fertility

CONCLUSION

The present study is revealed that recycling of degradable organic pulse waste and its safe disposal by using vermicomposting obtained with manure for utility of plant growth. It was found from the experiment that the suitable rate of casting released in less proportions such as 20% and 40% of pulse wastes whereas increasing in percentage retarded the rate of vermicompost reason for the risk in survival of earthworm *E. foetida*. Vermicomposting is the process of activation of earthworm serves as nature's gift to produce good humus, which is the most precious material to fulfill the needs of crops by providing all essential nutrients and growth promoting hormones through microbes.

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Table. 1 Colour and odour of vermicompost in all proportions after completion of Vermicomposting

Sl.No.	Observations	Colour of vermicompost	Odour of vermicompost
1	Control	Dark brown	Odourless
2	Set-1	Dark Black	Odourless
3	Set-2	Dark Black	Odourless

4	Set-3	Black	Odourless
5	Set-4	Not obtained	Odour
6	Expt.	Not obtained	Odour

Table. 2 Measurement of Physico-chemical parameters of various observations

Sl. No	Observation	Electroconductivity in S/m			PH			Moisture content			Temperature in ^{0}C	
		Initial	Final	Difference	Initial	Final	Difference	Before vermicomposting	After vermicomposting	Difference	Initial	Final
1	Control-100%	0.625	0.611	0.014	7.1	6.8	0.3	0.357	0.594	0.237	32.6	28.0
2	Set-1	0.890	0. 872	0.018	6.7	6.9	0.8	0.270	0.384	0.114	30.8	28.2
3	Set -2	0.897	0.872	0.025	6.7	6.1	0.6	0.321	0.415	0.094	31.7	28.4
4	Set -3	1.360	1.337	0.023	7.6	6.9	0.7	0.489	0.5	0.011	30.6	29.1
5	Set -4	1.878	1.877	0.001	7.5	7.4	0.1	0.249	0.253	0.004	31.6	34
6	Expt	2.03	2.07	0.04	6.4	6.2	0.2	0.411	0.418	0.006	32.3	34.7

Table.3. Electro-Conductivity and moisture Parameter

Sl.No.	Observations	Initial number of Earthworm	Survival of earthworm in 15days	Survival in 30days/after 1 month	Survival in 45 days	Survival in 60days
1	Control	4	5	7	13	22
2	Set--1	4	4	5	9	15
3	Set--2	4	4	6	9	12
4	Set--3	4	4	4	3	0
5	Set- -4	4	2	1	0	0
6	Expt.	4	0	0	0	0

Table. 4 Growth of earthworms presented through Mean±SD before and after vermicomposting

Experimental setup	Length		Diameter		Perimeter	
	Mean	SD	Mean	SD	Mean	SD
Control-100%	3.23	0.22	0.23	0.16	0.62	0.36
Set-1	2.35	0.46	0.21	0.082	0.575	0.23
Set -2	2.23	0.92	0.28	0.216	1.6	1.34
Set -3	2.47	0.48	0.21	0.116	0.65	0.19
Set -4	2.93	0.88	0.225	0.093	0.75	0.32
Expt.	5.63	1.47	0.575	0.05	1.97	0.38

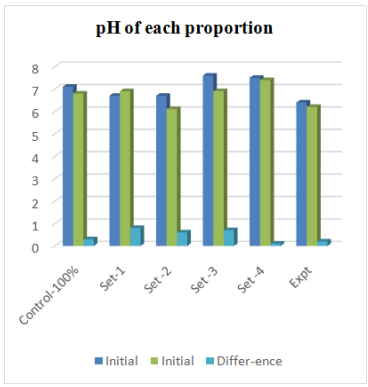

Fig.1 Graphical presentation of pH during during vermicomposting

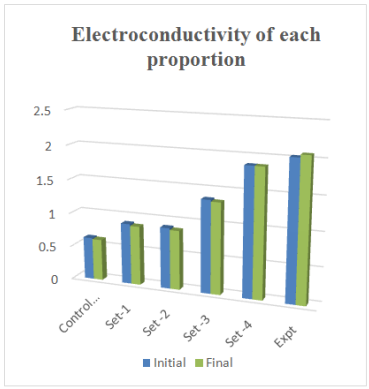

Fig.2 Graphical presentation of EC vermicomposting

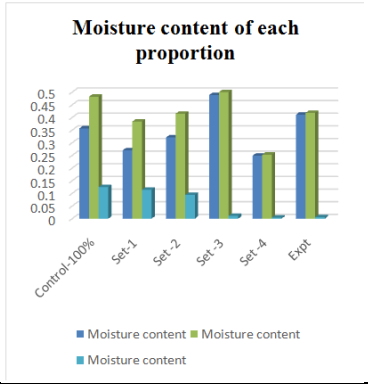

Fig.3 Graphical presentation of moisture vermicomposting

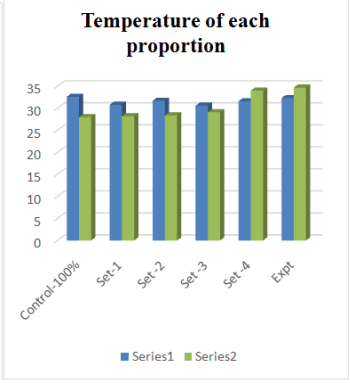

Fig.4 Graphical presentation of content during Temperature during vermicomposting

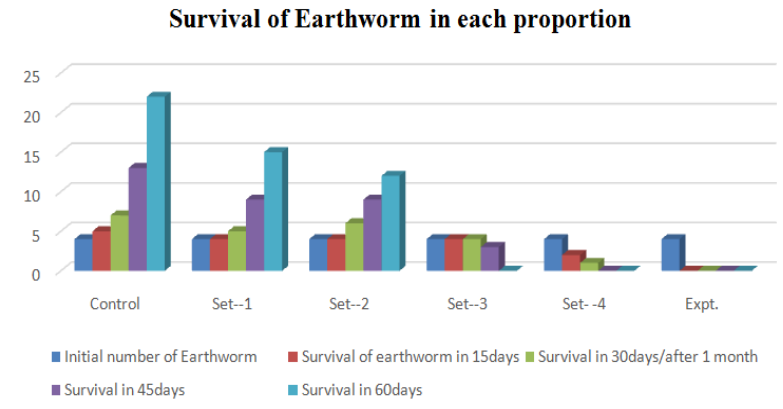

Fig.5 Survival of earthworms in each proportion during vermicomposting

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RESEARCH ARTICLE

A Study of Intelligent Crop Recommendation Systems in India by Analysing the Agricultural Soil

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ABSTRACT

The general population of India rely on agriculture for their means of support either directly or indirectly. In the developing countries such as India, agricultural farms are the most effectively utilized areas. Thus the chief supporter of Indian economy is agriculture as it performs a convincingly major role. In spite of this circumstance, a proper structure that recommend the farmers on prediction of the yield does not exist in the country. They are completely reliant on their own perception and without recognizing that the yield is an aspect that varies depending on the condition of soil and weather, they are satisfied in going along with the old-fashioned methodologies. In this paper we analyse the need for intelligent agriculture by focusing on the soil health and the existing circumstances in India for implementing soil health awareness among the farmers.

Keywords: precision farming, soil attributes, soil health, and soil health card (SHC, agricultural soil).

INTRODUCTION

India is one of the ancient countries that practices agriculture by carrying out farming in an area of more than 1.6 million square-kilometers which is the second-highest area of land for agriculture [1]. The economy of India relies primarily on agriculture as majority of the people of India practices agriculture. The rural areas of the country progresses by the elevation achieved in the economy by agriculture. The food security of a country is realised by the efficient usage of the agricultural lands. In spite of the primary need for agriculture in India, for years together, several aspects are infecting the strength of agriculture. Technology influencing approaches are practiced in much lesser manner. The farmers depend much on their own insight and they were completely following the older

practices. There are enormous attributes that influence the growth of the crops which cannot be taken in to consideration by a single farmer. Without getting the statistics on actual need, either abundance or shortage of production is resulted. The farmer's one single unwise decision might end up in detrimental consequences that not only affects him but also the economy based on agriculture [2]. Returns from the cultivation is hence not up to the expectation and this leads to quitting agriculture and turning in to different businesses by the farmers. However in the recent past, for recovering the lost strength of agriculture, several innovative technologies have evolved. One of the major enhancement that emerged in the field of agriculture is precision farming which is the technique of "site-specific" farming [3]. Utilisation of Satellites, Field sensors, unmanned flying engines (UAVs) supply us with enormous volume of data on climatic conditions, plant physiology, soil circumstances, water requirements, level of fertilisers, expected pesticides and still more [4]. The immediate necessity is to assist the farmers in taking decisions about the crucial factors of farming by developing a system which could offer analytical understanding. For the issues been faced nowadays by the world of agriculture, automation is the primary solution.

Many different factors of agriculture have to be considered for the process of automation and enrichment. One of the aspects is the concept of agriculture planning. The crucial attribute here is the crop selection which consequently would lessen the damages, in case any adverse situations happen. On the other hand if the circumstances are propitious, it would also make the most of the crop yield. For plant classification, identification of types of leaves, distinguishing soil categories, plant disease diagnosis and so on, computers are playing a successfully vital role [5]. The next essential factor of agriculture is soil. Every kind of soil has different type of qualities and different type of crops are cultivated in different kind of soils. In order to have a good understanding of what type of crops grow well in what kind of soil, the nature and quality of the soil types should be very well known to the farmers which is supported by the Machine learning techniques [6].

Soil Quality Attributes

Soil Health is the capability of a soil to perform within land use boundaries and ecosystem, to maintain environmental quality, endure biological productivity, and promote human, plant and animal health. The soil health parameters could be analysed discretely as physical, chemical and biological properties, however, the collaboration of these parameters produce and maintain a nutritious soil. More specific biological indicators of the soil, the corresponding methodologies employed and the major functions of the soil are as indicated in detail in the following table

Classification and pH Value of Soil

The quantity of the yield and its corresponding quality are the indispensable factors in the field of agriculture. The highly cherished resource that plays a major role in this aspect is the agricultural soil. Soils are compound blends of water, minerals, air, organic matter and innumerable organisms which are the decomposing residues of living things that lived once upon a time. It is termed as the "skin of the earth". The classification of soil could be based on its attributes such as permeability, stiffness and strength. Soil classification is very much essential for farming since based on the soil type crops are grown and also it assists the farmers to improve the soil by means of adding the necessary nutrients to the soil [7]. Following table provides a summary of the Indian standard soil classification system based on the size of the grain. The pH value of the soil determines the degree of acidity or basicity of the soil which in turn defines the obtain ability of the nutrients and the subsequent plant growth. The pH value of 7.0 is thought out to be neutral and the corresponding values that are greater than and lesser than this value are said to be in the nature of either acidic or alkaline [8] which is depicted in Fig 2.

The essential fragment of the environment is considered to be the soil and its qualities are primarily defined by the area of the land over which they have formed. Growth of the plants is absolutely affected by the soil acidity or alkalinity. The plant will not be able to absorb the nutrients such as phosphorus (P), potassium (K) and nitrogen (N),

under the condition that the soil is either too sweet or too sour. The quantity of organic matter and the occurrence of water and oxidation state of iron & magnesium determines the extensive variety of the colour of soil such as white, black, grey, brown, red and yellow [10]. Occurrence of iron oxide is the result of the yellow or red soil. Good level of organic matter results in dark brown or black colour in soil. Wet soil would be darker compared to dry soil. Oxidation instigates the red and brown colours. In addition to this, the existence of certain minerals also have an impact on the colour of the soil. Black colour is caused by manganese oxide, green colour by glauconitic, and white colour of soil in dry areas by calcite [11].

ALOGRITHMS TO CLASSIFY SOIL ATTRIBUTES

In order to determine which crops to grow on a specific soil and what are all the fertilizers needed, it is much vital to classify the soil. Following algorithms are employed for the classification function:

Naive Bayes

This classifier is a simple probabilistic classifier grounded on employing Bayes' theorem with robust independence assumptions. Naive Bayes classifiers could be trained much effectively in a supervised learning framework, based on the probability model's accurate nature. The key benefit of the naive Bayes classifier is that it has the need of only a lesser volume of training data for the estimation of the parameters such as means and variances which are needed for the purpose of classification [12].

J48 (C4.5)

Ross Quinlan developed this algorithm. It is an extension of Quinlan's previous ID3 algorithm. It is an open source Java implementation of the C4.5 algorithm in the Weka data mining tool. C4.5 is a program which generates a decision tree established on a collection of input data that are labeled. For quantifying about how satisfactorily it generalizes, this decision tree could then be verified against unseen labeled test data. C4.5 employs ID3 algorithm which is the cause for continuous attribute value ranges, rule derivation, pruning of decision trees, etc. C4.5 is frequently denoted as a statistical classifier since the decision trees spawned by C4.5 can be utilised for classification [13].

JRip

This algorithm proposed by William W. Cohen is an optimized version of IREP and it implements a propositional rule learner, Repeated Incremental Pruning to Produce Error Reduction (RIPPER). J-Rip classifier is one of the decision tree pruning models which is based on the rules of association. It is an efficient methodology for reducing error pruning and here the training data is fragmented into two sets and with the support of pruning operators the error is lessened on both the sets. Conclusively, the rules are developed from two sets which are defined as Growing set and Pruning set [14].

CREATING FUNCTIONAL SOIL DATABASES

Digital Soil Mapping (DSM) an essential term in soil sciences meant for incorporates various methodologies of offering soil relevant digital data. It provides clarifications for an emerging requirement of world-wide high resolution soil maps and the possibilities provided by spatial modelling and modern GIS analysis. A notion of Geomorphographic Maps (GMK) which are the entities with homogeneous conditions on the subject of the terrain, that is absolutely independent of scale and appropriate for countrywide surveys, was established. A procedural structure for the spatial prediction of the distribution of soil characteristics with the aid of improved terrain parameters, supposed to be the process parameters, was organised further to be utilised for large and medium scale research. In contradiction to the standard approaches for classifying mapping, soil characteristics are construed functionally as different continuous metric values. Various procedures – statistical, geostatistical and combined – to generate these metric soil data were tested and developed further. These methods necessitate digital elevation

models, climate modelling data, metric data of soil characteristics derived from soil profiles, remote sensing data, etc. and the local knowledge of regional soil and landscape genesis.

Geological maps could offer essential clues on the parent material of soil genesis. The difficulty is that we won't be able to attain homogeneous data sets pertaining to the scale of the underlying map in case several countries are participating. Frequently the geological strata are allocated to national bio- and lithostratigraphies, which do not fit in detail. The only technique to assimilate the information are either by handling the polygons of the geologic vector data as process areas or by using them for further interpretation of the entities of the geomorphographic data as the result of the terrain analysis process

PREVAILING SOIL HEALTH CONDITIONS IN INDIA

In accordance with the report submitted recently by a consortium of agriculture institutes, out of 350 million hectares of soil in India, 120 million hectares has turned already problematic, in the sense that the soil has turned acidic, saline, or alkaline. With the upsurge in salinity, the carbon content in the soil is discovered to be lessening. Paired with the surplus dosing of fertilizers which influences the nutritional value of the soil, the report advises that the country is on the brink of a soil crisis which in turn would have a huge impact on agriculture productivity, sustainability and on human health too [19]. Soil health being an aspect of physical, chemical and biological processes is persistently deteriorating and is quoted often as one of the causes for the crop yields being idling or worsening. For a balanced production in the crops, it is indispensable for all the related ones to be familiar with the soil environment in which plants grow on the whole, to be aware of the challenges of that environment and to restructure wherever feasible without breaking the soil quality. The degradation of soil together with insufficient and imbalanced application of nutrients and abandonment of organic manures is the source of multi-nutrient deficiencies in several locations. Together with this, inadequate field water management is the primary reason for low yield and reduced nutrient. It remains as a biggest risk to the soil's essential capacity to withstand production for future generations. The key concerns in association with the fading soil health are as listed below:

Soil erosion

The loss rate of soil in our country annually is around 15.35 tonnes per hectare, bringing about a loss of 5.37 to 8.4 Million tonnes (Mt) of nutrients, which in turn lead to the decrease in productivity of crops, happening of floods/droughts, decrease in capacity of the reservoirs (1 to 2% annually), and loss of biodiversity (Sharda and Ojasvi, 2016).

Physical degradation

For the crop production, the soil health physical deterioration because of water logging, flooding, submergence, crusting, soil compaction, impedance to root penetration and poor infiltrability grow into restrictive issues. About 1.07 Mhaarea is currently under physical degradation

Chemical degradation

Chemical degradation of soil health occurs because of:

i) acidification
ii) salinization/alkalinization
iii) soil toxification by chemicals
iv) running down of nutrients and organic matter and other nutrient input associated problems.

Around 6.74 Mha are under salt affected soils comprising of 3.79 Mha under high sodicity (pH > 9.5) and about 3 Mha (including 1.25 Mha coastal salinity) are under salinity (NAAS, 2010a). As shown in the following fig, the micronutrient deficiency in crops is rising swiftly both in intensity and extent and according to the analysis carried

out under All India Coordinated Research Project on 'Micro and Secondary Nutrients and Pollutant Elements in Soils and Plants', nearly 49, 15, 6, 8, 11 and 33% samples were found to be deficient in zinc, iron, manganese, copper, molybdenum and boron, respectively, across the country and thus all add on to the poor soil health (Shukla et al., 2012).

Biological degradation

Biological degradation of soil health crop up because of soil erosion by water leading to the loss of fauna and flora, abandonments of acidity and alkalinity, loss of organic carbon, extreme usage of chemicals, accumulation of toxic substances, extremes of climate, rigorous cultivation, etc. The size and complexity of soil communities are also reduced due to the management procedures that degrade organic matter in soils, or bypass biologically-mediated nutrient cycling. Soil organisms, including both animals and plants are significant for preserving the overall soil quality, stability and fertility.

CREATING AWARENESS AMONG FARMERS

The Soil act as the essential element of ecosystem functions that supports the food production, forestry products and human health. Healthy crops are produced by the healthy soils which in turn nurture people and animals. Among the soil properties, soil health plays the foremost part in agricultural productivity, environmental resiliency, food quality, and ecosystem sustainability. The present attention in soil health echoes the emerging awareness about soil as a fundamental constituent of the biosphere. It is projected that soil health is reliant on the upholding of four main functions: carbon transformations, nutrient cycles, soil structure maintenance and the regulation of pests and diseases (Kibble white et al., 2007). The usage of fertilisers in a non-judicial way, lesser application of organic matter and non-replacement of exhausted micro and secondary nutrients over the years have brought about nutrient deficiencies in soil.

Hence the Government of India has commenced a new scheme of offering Soil Health Card to farmers. The soil health card (SHC) provides data on soil health so that they can acquire proper guidance on the effective utilisation of fertilizer to cultivate crops based on soil health assessment that contains useful data on soil based on chemical analysis of the soil to describe soil health in terms of its nutrient availability and its physical and chemical properties (Mukati et al., 2018). The Soil Health Card System brings together the scientific community in the field of agriculture, techniques and cropping practices, the information repository of most modern tools, the farmers and the Government for the economic prosperity of the people (Patel,2013). An attempt was taken to assess the awareness and various limitations undergone by the farmers in making use of the information for an enhanced employment of recommended dose of fertilizers. The study was carried out in Kolhapur district of Maharashtra State. Awareness about soil health card and its information was analysed as shown in the following table. From the data collected, it was clear that majority of farmers (95%) were aware about the soil health card and they are certain that it offers information on the status of nutrients existing in the soil and that they deliver remedial measure for recuperating soil health and for getting better yield (82%) [22].

CONCLUSION

Agriculture plays a lead role in India. Wealth of the nation is determined by the welfare of the farmers. Soil plays a key function in the social and economic development by making certain the food, fodder and renewable energy supplies to endure human, animal and plant life. The prevalent issue prevailing amongst the Indian farmers is that they are not selecting the right crop based on the requirements of their soil which in turn leads to a life-threatening setback in productivity. Also our driving force to produce more, has pushed to misuse the soil that consecutively have an effect on the state of our health and well-being. This paper addresses the need for intelligent agriculture by maintaining the soil health.

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Table 1. Soil biological indicators, methodologies, related main soil functions [17]

Indicator	Methodology	Main soil functions
Individual, population and community level		
Presence, richness, abundance of individual soil organisms (for details see Supplementary Table 6)	Traditional handsorting and microscopic methods; molecular quantitation (qPCR).	Element, organic matter and water cycling, biological population regulation, soil structure maintenance
Microbial biomass and fungal biomass, fungal:bacteria ratio	Direct counting, chloroform fumigation extraction, SIR, PLFA, molecular quantitation.	Element and organic matter cycling, decomposition, soil structure maintenance
Indices based on faunal communities (e.g. Maturity Index, Enrichment Index, Channel Index, Structural Index for nematodes)	Counting and identification of specific groups of organisms	Element and organic matter cycling, biological population regulation, decomposition
Community composition	Manual counting and identification	Element and organic matter cycling, biological population regulation, habitat provision, decomposition, soil structure maintenance
	PLFA	
	Fingerprinting methods (e.g. DGEE, T-RFLP, A-RISA, ARDRA, TGGE), microarrays	
	Sequencing (metabarcoding)	
	Community Level Physiological Profiling (Biolog™, MicroResp™)	Element and organic matter cycling, decomposition, habitat provision
Ecosystem level		
Soil respiration, nitrogen mineralization, denitrification, nitrification	CO_2 evolution, N_2O emission, NO_3 produced.	Element, organic matter and water cycling, decomposition, habitat provision
Potentially mineralizable nitrogen	Anaerobic incubation.	
Metabolic quotient (qCO_2), microbial quotient (MicrC/SoilC)		
DNA and protein synthesis.	Thymidine and leucine DNA incorporation.	
Enzymatic activities	Extraction of enzymes in the soil and incubation with various substrates.	Element and organic matter cycling, decomposition,, biological population regulation
Functional genes and transcripts	FISH, Microarrays, *meta*-transcriptomic, qPCR, metagenome analysis.	
Metabolomics and metaproteomics	Assessment and quantitation of metabolites and proteins in the soil.	Element and organic matter cycling, decomposition,, biological population regulation, soil structure maintenance
Stable isotope probing	Incorporation of ^{13}C- or ^{15}N-labelled substrates into DNA, RNA, PLFA, proteins	Element and organic matter cycling, decomposition

Table 2. Classification of soil based on size of grains [7]

Very coarse soils	Boulder size		> 300 mm
	Cobble size		80 - 300 mm
Coarse soils	Gravel size (G)	Coarse	20 - 80 mm
		Fine	4.75 - 20 mm
	Sand size (S)	Coarse	2 - 4.75 mm
		Medium	0.425 - 2 mm
		Fine	0.075 - 0.425 mm
Fine soils	Silt size (M)		0.002 - 0.075 mm
	Clay size (C)		< 0.002 mm

Table 3. Analysis on awareness of soil health card information [22]

Awareness about soil health card	**Yes**	**No**
Soil health card provides information regarding the status of available nutrients(Macro & Micro) in the soil	57 (95%)	03 (05%)
Soil health card provide corrective measures a farmer should take for improved soil health and for better yield	49 (82%)	11 (18%)
Soil health card helps farmers in reducing extra expenditure by supplying required nutrients in the soil	53 (88%)	07 (12%)
The soil health card helps the farmers to get an idea on the crop wise recommendation of nutrients and fertilizers required in each type of soil.	48 (80%)	12 (20%)
Soil health card can be helpful and effective only if the recommendations are followed by farmers on regular basis	43 (72%)	17 (28%)
The technical information provided in soil health card has been made available in local language	47 (78%)	13 (22%)
Soil health card helps in practicing farming in scientific way	50 (83%)	10 (17%)
Soil health card helps to check the excessive use of fertilizer	45 (75%)	15 (25%)
Soil health cards provides clue to health of farm and its strength and weakness in terms of different nutrients and organic carbon ingredients	55 (92%)	05 (08%)
Number of crops increased in one year after soil testing	51 (85%)	09 (15%)
Expenditure of crop production decreases after soil testing	52 (87%)	08 (13%)

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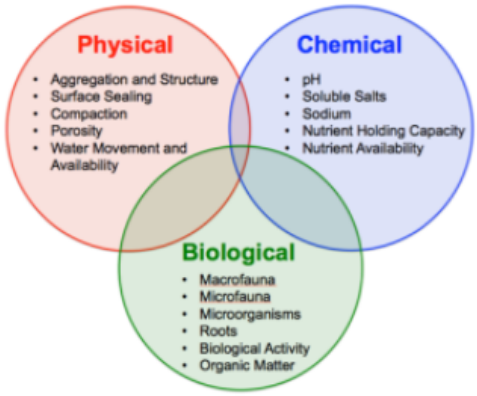

Fig 1. Parameters that determine soil quality [16]

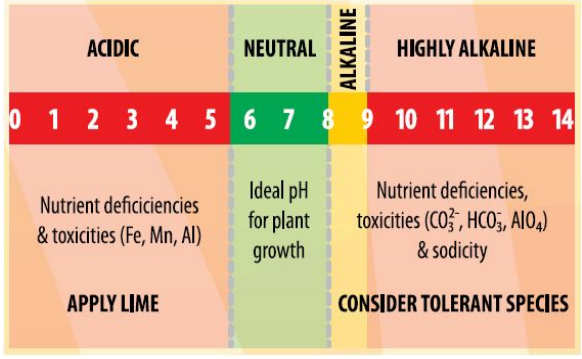

Fig 2. Classification of soil based on the value of pH [9]

Fig 3. India's soil health requires attention. (Source: IWP Flickr photo)

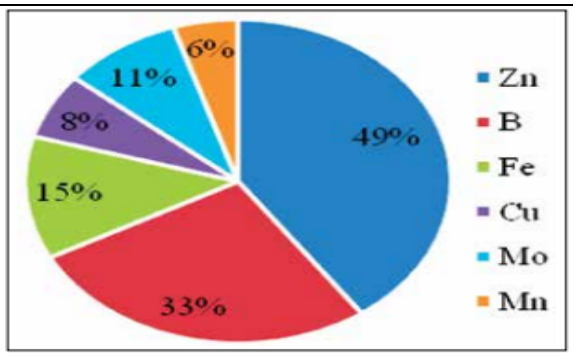

Fig 4. Deficiency of Micronutrients in Indian soils [20]

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RESEARCH ARTICLE

Comparative Analysis of the Energetic Diffusion in the IGT-Chalcogenide Glasses

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ABSTRACT

The composition dependence of energetic parameter (A) in two sets of IGT (Ge-Te-In) glasses, namely, $(GeTe_4)_{100-x}In_x$ (x = 0, 5, 10, 15, 20) and $(GeTe_5)_{100-x}In_x$ (x = 0, 5, 10, 15, 20) have been studied by using rigidity percolation theory and bond constraints theory. Parameter (A) shows a local maxima at <r> = 2.52. The results are discussed on the basis of the topological and rigidity theory exhibited by covalent network glasses. The relative sensitivity of parameter (A) to these phenomena discussed. The variation of energetic parameter with mean coordination and energy gap also discussed.

Keywords:-IGT (Ge-Te-In) Chalcogenide glasses, Heat of atomization, Energy gap and Energetic parameter.

INTRODUCTION

Germanium chalcogenide (Ge-Chal) glasses are interesting material for infrareds optics. They have a large range of transparency and good mechanical properties such as hardness, adhesion and internal stress [1]. Infrared optical Fibres operating at 2-12µm in wavelength are required for infrared sensing applications such as radiometric thermometry and CO_2 laser power applications[2] The Te-based chalcogenide glasses are used for such applications because their infrared absorption edges are located in a wavelength region above 12µm [3-5]. Chalcogenide glasses have aroused considerable interest in recent time because of their applications in Non-Volatile RAM. These materials

are also called phase change materials because the high resistance semiconducting phase is amorphous while the low resistance phase is crystalline. Binary chalcogenides such as As-Te, Al-Te and Ga-Te undergo a continuous transition from semiconductor to metal chalcogenide glasses such as Ge-Te, exhibit sharp discontinuous glassy semiconductor to crystalline transition [6-7]. Ge-Te and ternary alloys based on Ge-Te are currently of interest as phase change materials for reversible optical storage due to their structure dependent optical properties. Phase change materials have to be stable in both crystalline and amorphous phases and to switch reversibly between the two phases – amorphous and crystalline. The exploration of new fast switching and stable phase change materials is of scientific significance [8-10].

Indium doped GeTe glasses referred as IGT glasses has been recently proposed as promising candidates for phase change memories, as they are thermally more stable having a crystallization temperature which is about 150^0C higher than that of the commercially used GST (Ge-Sb-Te) sample; they also have a retention life of 10 years at temperatures higher than 150^0C. In PCM operations IGT is capable of achieving high thermal stability in amorphous state with high crystallization speeds [11-16]. In the present work we have reported the theoretical prediction of the compositional effect on the spectral dependence of the absorption or energetic diffusion in $(GeTe_4)_{100-x}In_x$(x = 0, 5, 10, 15, 20) and $(GeTe_5)_{100-x}In_x$ (x = 0, 5, 10, 15, 20) glassy systems. An attempt has been made to explain the varying trends of various parameters with increasing In content.

RESULT AND DISCUSSION

We studies mean coordination number of Ge-Te-In chalcogenides with constant Ge/Te ratio 1:4 and 1:5and various In content 0, 5, 10, 15, 20 at %.Parameters such as optical energy gap (E_g), Heat of atomization (H_s) and Energetic parameter (A) are defined.

Structure of Chalcogenide Glasses

Models based on chemical ordering [17] and network topology [18-21] has been proposed to explain the composition dependence of physical properties. The chemically ordered network (CON) model favors the formation of hetero-polar bonds and thus the glass structure is composed of cross linked structure units of stable chemical compounds and excess, if any, of the elements. It has been argued that chemical ordering leads to a chemical threshold at which specific features in the composition dependent variations occur [22]. The topological model is based on balancing the number of operative constrains with the number of degree of freedom. This model describes the composition dependence in terms of the average coordination number <r> and predicts a topological threshold at <r> = 2.4, where the rigidity of the network percolates [23]. The network is floppy below <r>=2.4 and rigid above <r> = 2.4. A later modification of this model [24] based on the formation of two dimensional layer structures and medium range interactions suggests a topological threshold at <r>= 2.67 where a change from two dimensional layered structure to three dimensional network takes place due to cross-linking. The applicability of the ideas of rigidity percolation was verified in many binary and ternary glasses [25-26]. Signature of rigidity percolation has been reported to occur at 2.4 in various glasses. In this paper we present results on the composition dependence of optical energy gap in two sets of IGT glasses, namely $(GeTe_4)_{100-x}In_x$(x = 0, 5, 10, 15, 20) and $(GeTe_5)_{100-x}In_x$(X = 0, 5, 10, 15, 20) glassy systems. The composition range covers the threshold composition predicted on the basis of various models .In terms of average coordination number <r>, calculated using Tanaka equation--

$$\mathbf{<r> = X_aZ_{Ge} + X_bZ_{Te} + X_cZ_{In}}$$

Where Z_{Ge}= 4, Z_{Te}= 2 and Z_{In}= 4 are the coordination number of Ge, Te and In respectively the compositions fall in the range 2.34 < (r) <2.52. The calculated values of <r> for $(GeTe_4)_{100-x}In_x$ (x = 0, 5, 10, 15, 20) and $(GeTe_5)_{100-x}In_x$(X = 0, 5, 10, 15, 20)glassy systems are listed in Table-1 and Table-2.

Heat of Atomization (H_s) and theoretical optical band gap (E_g)

Loffe and Regel [27] suggested that the bonding character in the nearest neighbor region which means that the coordination number characterizes the electronic and optical properties of the semiconductor materials. In terms of coordination number optical band gap is defined by-

$$E_g = a\,(H_s - b)$$

Where a and b are the adjustable parameters which depends on coordination number as-

$$b = 0.089(\langle r \rangle)^2$$

and H_s is the average heat of atomization which in terms is correlated with the energy of the isostructural systems. For ternary and higher order semiconductor compounds, the average heat of atomization H_s is defined for the compounds $A_\alpha B_\beta C_\gamma$ as a direct measure of cohesive energy and average bond strength is given by-

$$H_s = (\alpha H_s^{Ge} + \beta H_s^{Te} + \gamma H_s^{In}) / (\alpha + \beta + \gamma)$$

Where α, β and γ are the mole fractions of Ge, Te and In. H_s^{Ge}, H_s^{Te} and H_s^{In} are the heat of atomization of Ge(377kJ/mol), Te(197kJ/mol) and In(243kJ/mol) respectively. The calculated values of theoretical band gap energy are listed in Tables-1 and Table-2 and for the two compositions of Ge-Te-In.

The Energetic Parameter

The spectral dependence of the absorption coefficient indicates an indirect allowed transition. The result is verified by energetic parameter relation versus composition. According to Angell proposal [28], the compositional changes in the optical gap is correlated by energetic parameter (A) which is given by the equation-

$$A = \epsilon \Delta E_g / k$$

Where $\epsilon = \delta\,(z-2)$ and k = Boltzmann constant (1.38×10^{-23} J/Kelvin), δ is an independent constant (0.55).The variation of energetic parameter (A) as function of average coordination number <r> for the two sets IGT glasses studied are given in Fig.- 1. It can be inferred that the two sets of IGT glasses show identical trends in the <r> dependence. Energetic parameter (A) increases initially <r> is increased as well as Te. An explanation of the observed behavior can be given in the framework of the energy band model for the Chalcogenide glasses proposed by Kastner[29] and the change in the average bond energy of the system as the composition varied. According to the Kastner the valance band in the chalcogenide glasses is constituted by the lone-pair bands where the conduction band arises from the anti-bonding band. In a multi-component glass like Ge-Te-In the position of conduction and valance band edges and thus the energy gap largely depends on the relative number of various possible bonds in the system and the average bond energy. The various possible bonds in the Ge-Te-In system are Ge-Te, Ge-Ge, Ge-In and Te-In. The bond energies are 200.02Kj/mol, 185Kj/mol, 146.06Kj/mol and 217.97j/mol respectively. The values of average coordination number<r>, energy gap (E_g) and energetic parameter (A) for the two glassy systems are listed in Table:-1 and Table: -2 The observed initial increase in E_g with increase in <r> suggests that in this region of curve the influence of the relative number of Ge-Te and Te-In bond are less prominent in determining the band gap than that of the $GeTe_4$ structural unit. It appears that Ge-Te bonds are present in all these compositions. The increase in E_g continuous under the influence of the relative decrease in the number of strong Ge-Te bonds.

CONCLUSION

The variation of energetic parameter (A) of the two sets of IGT glasses studies show features at different <r> values. These results can be interpreted as a signature of two different phenomena occurring in this system, namely, the chemical threshold and the topological threshold. Since E_g is more sensitive to variations of the relative number of different bonds and the average bond energy of the system. The variation of Eg and A shows tendency of increase when In content increasing . This behavior is expected because In atom has bigger covalent radii (CR) then Ge and Te atoms (Ge_{CR} = 1.22Å,Te_{CR} = 1.35Å and In_{CR} = 1.44Å)Anomalies that occur in energetic parameter (A) of $(GeTe_4)_{100-x}In_x$ from 10% In content and from 15% In content for$(GeTe_5)_{100-x}In_x$ corresponding to the average coordination number 2.43 and 2.40 expand the re-arrangement of the structure occurs transforming from floppy to rigid structure and the atomic bound becomes tighter and shorter.

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Table.1: Value of <r>, E_g and A for $(GeTe_4)_{100-x}In_x$

X at%	compositions	<r>	E_g(eV)	A x 10^4/kel.
0	$Ge_{20}Te_{80}$	2.40	2.4097	1.3522
5	$Ge_{19}Te_{76}In_5$	2.43	2.4145	1.4627
10	$Ge_{18}Te_{72}In_{10}$	2.46	2.4193	1.5890
15	$Ge_{17}Te_{68}In_{15}$	2.49	2.4240	1.7003
20	$Ge_{16}Te_{64}In_{20}$	2.52	2.4288	1.8275

Table.2: Value of <r>, E_g and A for $(GeTe_5)_{100-x}In_x$

X at%	compositions	<r>	E_g(eV)	A x 10^4/kel.
0	$Ge_{17}Te_{83}$	2.34	2.3518	1.1097
5	$Ge_{16}Te_{79}In_5$	2.37	2.3567	1.2022
10	$Ge_{15}Te_{75}In_{10}$	2.40	2.3615	1.3251
15	$Ge_{14}Te_{71}In_{15}$	2.43	2.3662	1.4485
20	$Ge_{13}Te_{67}In_{20}$	2.46	2.3710	1.5724

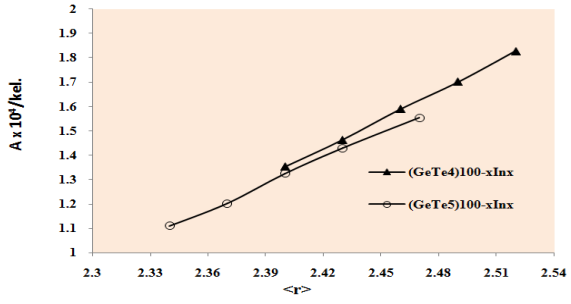

Fig.1: Variation of Energetic parameter (A) with average coordination number <r> for the two sets of Ge-Te-In Glasses

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RESEARCH ARTICLE

Amelioration of Aluminium Toxicity by Calcium: Evaluated in Conventional and Modified *Allium* Test

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ABSTRACT

Allium test is the oldest and the most reliable of the different tests to evaluate the toxicities of metallic compounds. As the conventional *Allium* test suffers from a number of drawbacks it was modified to make the assessments even more reliable. The self-devised modified test was than compared with the conventional method taking various parameters subjecting the plant to Al-induced toxicity in acidic medium. Further, it was employed to study the ameliorative nature of calcium compounds on the Al-induced toxicity symptoms, wherein, three different salts of calcium at fixed doses were mixed with aluminium chloride solutions to find out what works best to alleviate the level of aluminium-induced toxicity measured through decrease in length of roots of onion bulbs. It was found that all the salts did alleviate the toxic effect but $CaCO_3$ was much more effective than that of $CaCl_2.2H_2O$ and $CaSO_4.2H_2O$. The reason for higher efficacy of $CaCO_3$ could be due to its alkaline nature when in solution, which decreases aluminium availability to the roots. The tests using local underground water with high calcium content revealed good alleviation of the induced toxicity levels presumably for the same reason. Hence, application of calcium carbonate in the form of lime in the affected soil and /or use of underground water for irrigation may help contain the emerging problem of aluminium toxicity in this locality.

Key words : Aluminium, aluminium toxicity, *Allium* test, Calcium, amelioration, remediation

INTRODUCTION

Phyto-toxicity of elevated aluminium in acid soil is well documented[1,2] limiting crop production globally[3]. Of the different methods of measuring toxicity to higher plants, the root elongation test is the most sensitive for metal ions [4]. Such a test, therefore, becomes more appropriate/significant for Al^{3+} since it is known primarily to be rhizotoxic[5,6,7] lessening root elongation within 5 minutes through its binding tightly to the cell wall and checking loosening[8].

Allium test is employed to find out relative toxicity of metal ions[9,10] and pharmaceutically active compounds[11] including nanoparticles[12]. Positive results in the *Allium* test is considered a warning and also an indication that the tested chemical may be a risk to human health and to our environment[13] for the test has shown good correlation with other test systems, both in general toxicity (growth) and in genotoxicity (chromosome aberrations)[14]. Since its introduction [15], *Allium* test is done in test tubes with no or little modifications [16,17]. This method however, suffers from a number of constraints like anoxygenic condition in the test solution, regular loss of solution due to evaporation, non-availability of sufficient space for root growth. To obviate the above constraints there is a need to devise a better method for *Allium* test which can give a clear picture of toxicity assessment. An attempt has been made in this direction by devising a new set-up to measure the actual range of phyto-toxicity of aluminium (with the deviation from the conventional method if any). Further, through the modified *Allium* test, it was attempted to ameliorate the toxicity due to Al by the application of various calcium compounds and local tube-well water (pH 7.38; Total alkalinity, mgL^{-1} as $CaCO_3$ 451.00; calcium, mgL^{-1} 42.00) individually, also to know the role of pH of the exposure medium vis-à-vis the Ca:Al application, since Al^{3+} is solubilized into a phytotoxic form as the soil becomes acidic.[5]

MATERIALS AND METHODS

The Test Chemicals

$AlCl_3.6H_2O$
$CaCO_3$; $CaSO_4.2H_2O$; $CaCl_2.2H_2O$

All the reagents were of analytical grade. Double deionized water was used throughout. All growth solutions were prepared from stock solution of $AlCl_3.6H_2O$ (100 mgL^{-1}), and pH was maintained in the range of 4.5-5.0 wherever necessary adding drops of 1N HCl or NaOH to the experimental growth media.

Test Procedure

Small-sized healthy bulbs (about 15-20 mm diameter) of the locally available onion, *A. cepa* (2n = 16), were obtained from the market and the tunica was removed slowly. With the help of a new blade the dry lower portion of the discoid stem was also removed carefully without damaging the root primordia. The processed bulbs were placed over distilled water on self-devised set up at a maintained temperature range (20 ± 2 ◦C) at dark to initiate the root germination. As the roots attained 2-3mm in length, the bulbs were taken out, thoroughly washed with deionized water also the excess water was removed from the bulbs by using tissue papers before being used in the designed experiments.

Experiment 1. Conventional Method

Healthy processed bulbs were placed over glass tubes (15 X 150 mm) containing different concentration, 1 to 6 mgL^{-1}, of Al^{3+} as $AlCl_3.6H_2O$ in sets of 6 tubes (20 cm^3) for each. Distilled water treatment served as control. The whole set-up was kept in dark at 20 ± 2ºC. The test solutions and the distilled water control were changed every 24h. After 72h of exposure, roots from each bulb were counted and measured. Root length was estimated from whole root bundle and each bulb served as a sample with its own mean, range and deviation. The roots of each bulb were weighed afresh and then oven-dried (80ºC, 24h) to constant weight and dry weight was recorded.

Experiment 2. (Self--devised) New Set-up

Bulbs of uniform size were placed over a perforated/open base plastiware (6 cm dia. X 8 cm height) with three holes of 10 mm dia. each. The plastiware was placed inside a beaker (300 cm^3) containing 250 cm^3 of different test solutions as described earlier. Each such beaker served as a set with 3 bulbs in it. An aerator was fitted to supply air

continuously through a common plastic tube with separate regulators for each set. Further, the beaker had a knobbed-outlet to drain the solution out of the beaker as and when required without disturbing the bulbs which also eased the process of daily replacement of water/the solution of the set-up (Figure 1). For one experiment, 2 such set-ups were made and they were kept in dark at 20 ± 2°C for 72h followed by root (length and weight) measurements as described earlier.

Experiment 3. Growing Bulbs in Nutrient Medium

The same procedure was followed as in Experiment 2, but the distilled water was replaced by dilute Hoagland's nutrient medium. Pilot experiments indicated maximum growth of onion roots at 8% dilutions (pH 6.52).

Subsequent experiments were done to study amelioration by different Ca salts in three separate experiments (Experiment 4-6) following the method described in Experiment 2. In these experiments amelioration of around 50% of Al^{3+} toxicity was taken for the study using three Ca compounds viz. $CaCl_2.2H_2O$, $CaSO_4.2H_2O$ and $CaCO_3$ respectively.

In yet experiment (Experiment 7) local tube-well water was used in place of distilled water to find out a shift of the toxicity parameters for Al^{3+} if any.

Toxicity Parameters

LECT (Lowest Effective Concentration Tested): This refers to the treated concentration at which a stimulus begins to bring a measurable response. $MECT_{50}$ (Median Effective Concentration Tested): Here the reference is to the treated concentration which produces 50% of the specified response. HECT (Highest Effective Concentration Tested): It is the treated concentration that yields the maximum measurable effect of the specified response.

Cell Division Study

Following treatment, root tips were cut, washed and stored in ethanol-glacial acetic acid (3:1) fixative for 24 hours. To prepare root tip chromosome slides for calculation of MI and to study other cyto-toxic effects of Al, aceto-caramine squash technique was used. The fixed root tips were taken out and were hydrolysed in 1N HCl at 60°C for 4-5 minutes. The root tips were then transferred to deionized water and kept there for a few minutes and were transferred on a clean slide and stained with a few drops of 2% aceto-caramine and squashed under a cover slip. A minimum 2000 cells were scored for MI expressed as the percentage of total number of examined cells undergoing mitosis. The mean MI was the average of roots from each treated/control concentration of Al^{3+}. Obtained data were expressed as the mean ± SD of the means for MI. The same slides were also used later to record mitotic damages. Whole slides were studied to find out cells with spindle dysfunction, monitored by C-metaphases, vagrant chromosomes, star-shaped/ disturbed anaphases while clastogenicity was monitored by metaphase breaks, anaphase fragments, anaphase bridges and micronuclei in telophase or in interphase.[18,19]

Statistical Analysis

In all the experiments, the effects following the treatments at different Al^{3+}/Ca^{2+} concentrations were compared with each other following Least Significant Difference (LSD) test as per the procedure given by Gomez-Gomez [20]. For cell division effects, individual 2 × 2 contingent Chi-square test (χ^2) was carried out.

RESULTS

The effect of Al^{3+} in different doses brought about progressive decrease in the onion root length with the increase in the ionic concentration. The LECT value was 1.0 mgL^{-1} registering a retardation of ~15% growth compared to the

control. The $MECT_{50}$ value that brought about a reduction of ~50 % of control was less than 2.0 mgL^{-1}. The HECT value was 5 mgL^{-1} where the roots decreased up to 95 % of the control (Figure 2).

However, in the second experiment with the improved set-up, the values of LECT, $MECT_{50}$ and HECT were 2.5, ~7.25 and 15 mgL^{-1} of Al^{3+} respectively (Figure 3). In the third experiment the observed values of LECT, $MECT_{50}$ and HECT were 2.5, ~6.5 and 15 mg L^{-1} of Al^{3+} respectively (Figure 4). The set with different doses of $CaCl_2.2H_2O$ (Experiment 4) revealed that there is no significant amelioration up to a concentration of 200 mgL^{-1} of Ca^{2+} (Table 1). The set with $CaSO_4.2H_2O$ (Experiment 5) revealed that with a dose of 50 mgL^{-1} Ca^{2+} the % of injury to root length remained unaffected but with 100 mgL^{-1} Ca^{2+} it was brought down form about 65% to 42 % and with 200 mgL^{-1} Ca^{2+} the injury was reduced to about 35% of the control (Table 2). The set with $CaCO_3$ (Experiment 6) showed significant amelioration of Al-induced toxicity by different concentrations starting from 50 mgL^{-1} of Ca^{2+} onwards with complete recovery at 200 mgL^{-1} Ca^{2+} (Table 3). Experiment with tube-well water (Experiment 7) revealed that the $MECT_{50}$ value was shifted to ~100 mgL^{-1} of Al^{3+} (Table 4) from ~7.25 mgL^{-1} in the distilled water.

The effects of Al^{3+} on the cell division and chromosomes were monitored after 48 hours of growth of *A. cepa* bulb roots. It revealed a gradual fall in mitotic index (MI) and a gradual increase in percent of aberrations, the latter being significant from 5 mgL^{-1} onwards. The aberrations in the dividing cells were due to both spindle dysfunction and clastogeny. A few tetraploids among the dividing cells were observed (Table 5). Amelioration to the cytotoxic symptoms induced at 10 mgL^{-1} Al^{3+} on the cell division and chromosomes were studied using $CaCO_3$. It was found that the decrease in MI was gradually reversed with the increase in Ca^{2+} concentration from 50 mgL^{-1} onwards, with a full recovery at 200 mgL^{-1} of Ca^{2+}. Percentage of other induced cytological aberrations viz. spindle dysfunction, clastogeny and tetraploid cell formation were also decreased in the dividing cells with the increase in Ca^{2+} concentration (Table 6).

DISCUSSION AND CONCLUSION

The Test Procedure

The *Allium* test has been in use ever since 1938[20], used for the first time. This test got slightly modified[21] to test toxicity of environmental contaminations. However, some of the problems inherent with the test remained unaddressed or partly addressed[22] to especially for chronic treatments. These are the physical constraints imposed on the growth of roots by the test tubes, anoxygenic condition of the inside liquids and loss of liquids due to evaporation. The self-devised improved method was to obviate the above constraints. The results obtained were very different in the assessment of toxicity as reflected by a forward shift of doses of Al^{3+} in the toxicity parameters chosen for the study (Figure 3). This could give an idea about the actual toxic range of the metal barring other constraints. Next attempt was to measure the toxicity in the presence of a basal nutrient medium instead of distilled water to make the assessment more meaningful. However, the results did not show significant variations from the distilled water experiment (Figure 3 & 4). This suggests that the basic ions in the nutrient medium although recognized in plants as nutritional, they did not promote growth of the roots of onion at the tested concentration and duration. Therefore, amelioration tests were done in distilled water only in the three separate experiments for three different salts of Ca.

Study of Amelioration

Alleviation of Al^{3+} toxicity was attempted by many workers inter *alia* through chemical means e.g. silicon[23,24], boron [25], rock phosphate [26]. Calcium compounds are also known to alleviate toxic effects of aluminium [27,28,29,30]. However, the aluminium species present in soil solutions that are responsible for phytotoxicity are not clearly established[31] and reports are not univocal on a quantitative estimate of Ca:Al applicable to everywhere as well. The different parameters of measuring the toxicity provided useful guidelines for amelioration study with

application of different Ca compounds. For convenience, a dose higher to the $MECT_{50}$ value of Al^{3+} was taken to examine the differential extent of amelioration provided by the salts at fixed doses of Ca (from 50 to 200 mgL^{-1}) with three different anions. The results did differ and the order of efficacy was $CaCO_3 > CaSO_4.2H_2O > CaCl_2.2H_2O$ (Table 1-3). Not discounting the positive role of calcium, the differential response seems to have a bearing on the pH of the medium since pH is known to affect the availability of Al^{3+} to the roots[32] but not restricting the growth of *Allium* roots between pH 3.5 to 11[21]. The results did show that amelioration by Ca^{2+} appears more in high pH condition which hinders availability of Al^{3+}, which was the case with $CaCO_3$. Experiment with local tube-well water showed the $MECT_{50}$ value was shifted to as high as 100 mgL^{-1} of Al^{3+} (Table 4). This result is perhaps due to both high Ca^{2+} in water and the general alkaline medium. This suggests that the problem of Al^{3+} toxicity can be contained by application of Ca-salts which will have high pH in the medium. The experiments on the effects on dividing cells and chromosomes clearly indicate the action of Al^{3+} at different doses (Table 5). But the application of $CaCO_3$ did start reversing the effects even at a dose of 50 mgL^{-1} of calcium (Table 6). This was possible because of low availability of Al^{3+} with the increase in the pH of the medium. This study further corroborates the amelioration study on root elongation test. It is reported that under conditions of sub-soil acidity, conventional surface liming is of little use, in most of the cases, because of low mobility of alkaline component of lime [31]. In the present context the use of tube-well water can take care of the sub-soil acidity also. However, how exactly Ca protects the roots against Al^{3+} toxicity is yet to be known. The exact nature of this Ca-Al interaction seems worthy of future study[33] and current literature does not throw much light on this aspect.

The state of Odisha being largely agricultural and with acid soil condition [34] needs to address the emerging problem of Al toxicity. Application of calcium carbonate (in the form of lime) in the affected area may be tried to check Al^{3+} toxicity. But the quantity of lime has to be worked out for different environmental and agro-climatic conditions as well as the crop plants under cultivation. The use of tube-well water with high Ca^{2+} content (high pH) in irrigation should find good result.

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TABLE 1. Alleviation of Al^{3+} induced toxicity to Allium roots by Ca^{2+} ($CaCl_2.2H_2O$) after 72h of growth (New Set--up)

		Solution pH							
		Before Expt.	After Expt.	*Mean no. of Root ± S.D. per bulb	Range of root length (mm)	Mean root length ± S.D. (mm) (% of injury)	Mean root fresh wt±S.D. (mg) / bulb (% of injury)	Mean root dry wt ± S.D. (mg) / bulb (% of injury)	% of root length growth as compared to control
Control		4.55	6.22	22.83 ± 2.4 a	5 - 51	34.59 ± 3.6 (0) a	233.56 ± 28.7 (0) a	27.07 ± 2.1 (0) a	100
Al^{3+} 10 mgL^{-1}		4.68	4.50	10.61 ± 1.2 b	3 - 29	11.22 ± 1.3 (67.26) b	80.16 ± 8.8 (65.68) b	11.22 ± 1.0 (58.55) b	32.44
Al^{3+} 10 mgL^{-1} +	Ca^{2+} 50 mgL^{-1}	4.49	4.66	11.44 ± 1.0 b	2 - 32	10.45 ± 0.7 (69.79) b	78.92 ± 9.1 (66.21) b	8.84 ± 0.6 (68.09) b	30.21
	Ca^{2+} 100 mgL^{-1}	4.56	4.54	13.19 ± 0.9 b	3 - 43	10.64 ± 1.1 (69.24) b	86.48 ± 9.6 (62.97) b	9.23 ± 1.2 (66.68) b	30.76
	Ca^{2+} 150 mgL^{-1}	4.71	4.46	13.27 ± 0.8 b	3 - 38	12.60 ± 1.4 (63.57) b	104.52 ± 14.1 (55.25) b	12.09 ± 1.4 (55.35) b	36.43
	Ca^{2+} 200 mgL^{-1}	4.49	4.57	14.75 ± 1.9 b	5 - 46	13.81 ± 1.6 (60.08) b	102.44 ± 1.7 (56.14) b	12.84± 1.3 (53.65) b	39.92

*Mean value of root from 6 bulbs growing in two plastiwares.
Any two means having a common letter are not significantly different at 5%P level.

TABLE 2. Alleviation of Al^{3+} induced toxicity to Allium roots by Ca^{2+} ($CaSO_4.2H_2O$) after 72h of growth (New Set--up)

		Solution pH		*Mean no. of root ± S.D. per bulb	Range of root length (mm)	Mean root length ± S.D.(mm) (% of injury)	Mean root fresh wt ±S.D. (mg) / bulb (% of injury)	Mean root dry wt ± S.D. (mg) / bulb (% of injury)	% of root length growth as compared to control
		Before Expt.	After Expt.						
Control		4.59	6.15	24.52 ± 2.8 a	5 - 49	36.51 ± 3.8 (0) a	253.23 ± 31.2 (0) a	24.75 ± 3.1 (0) a	100
Al^{3+} 10 mgL^{-1}		4.54	4.74	11.25 ± 1.3 b	3 - 28	12.62 ± 1.8 (65.43) b	100.14 ± 9.6 (60.45) b	10.69 ± 0.8 (56.81) b	34.57
Al^{3+} 10 mgL^{-1} +	Ca^{2+} 50 mgL^{-1}	4.52	4.66	13.39 ± 1.4 b	3 - 31	12.99 ± 1.5 (64.42) b	109.58 ± 17.4 (56.73) b	10.72 ± 1.6 (56.69.) b	35.58
	Ca^{2+} 100 mgL^{-1}	4.61	4.58	15.37 ± 1.7 b	4 - 39	21.00 ± 2.4 (42.48) c	134.42 ± 17.1 (46.92) b	15.11 ± 1.5 (38.95) c	57.52
	Ca^{2+} 150 mgL^{-1}	4.55	5.14	16.24 ± 1.6 b	5- 46	22.93 ± 1.8 (37.20) c	146.66 ± 13.9 (42.08) c	15.74 ± 1.8 (36.40) c	62.80
	Ca^{2+} 200 mgL^{-1}	4.63	5.29	20.16 ± 2.4 a	3 - 48	23.77 ± 1.9 (34.89) c	168.38 ± 20.7 (33.51) c	18.24 ± 2.0 (26.30) c	65.11

*Mean value of roots from 6 bulbs growing in two plastiwares.
Any two means having a common letter are not significantly different at 5%P level.

Table 3. Alleviation of Al^{3+} induced toxicity to Allium roots by Ca^{2+} ($CaCO_3$) after 72h of growth (New Set--up)

		Solution pH		*Mean no. of root ± S.D. per bulb	Range of root length (mm)	Mean root length± S.D. (mm) (% of injury)	Mean root fresh wt± S.D. (mg) / bulb (% of injury)	Mean root dry wt ± S.D. (mg) / bulb (% of injury)	% of root length growth as compared to control
		Before Expt.	After Expt.						
Control		4.62	6.24	22.54 ± 2.2 a	7 - 55	32.42 ± 3.4 (0) a	249.29 ± 30.0 (0) a	24.61 ± 2.4 (0) a	100
Al^{3+} 10 mgL^{-1}		4.57	4.59	13.80 ± 1.5 b	3 - 24	11.24 ± 1.5 (65.33) b	101.56 ± 10.8 (59.26) b	11.05 ± 1.4 (55.10) b	34.67
Al^{3+} 10 mgL^{-1} +	Ca^{2+} 50 mgL^{-1}	6.00	6.33	20.33 ± 1.6 a	5 - 41	18.39 ± 1.7 (43.28) c	177.34 ± 20.3 (28.86) c	18.31 ± 1.3 (25.60) c	56.72
	Ca^{2+} 100 mgL^{-1}	6.56	6.70	20.81 ± 1.8 a	5 - 54	22.78 ± 2.3 (29.73) c	196.84 ± 21.1 (21.04) c	19.42 ± 2.1 (21.09) c	70.27
	Ca^{2+} 150 mgL^{-1}	6.88	6.68	25.27 ± 2.8 a	7 - 50	25.18 ± 2.2 (22.33) ac	222.73 ± 25.6 (10.65) ac	23.07 ± 2.5 (6.26) ac	77.67
	Ca^{2+} 200 mgL^{-1}	7.45	7.04	23.49 ± 1.2 a	8 - 56	33.05 ± 2.9 (Recovered) a	246.65 ± 28.4 (1.06) a	24.92 ± 2.5 (Recovered) a	98.86

*Mean value of roots from 6 bulbs growing in two plastiwares.
Any two means having a common letter are not significantly different at 5%P level.

Table 4. Effect of Al^{3+} as $AlCl_3.6H_2O$ on Allium on root growth after 72h of exposure in tube-well water (New Set—up.

Concentration of Al^{3+} in mg/Lt	Solution pH		*Mean no. of root ± S.D. per bulb	Range of root length (mm)	Mean root length ± S.D. (mm) (% of injury)	Mean root fresh wt ± S.D. (mg) / bulb (% of injury)	Mean root dry wt ± S.D. (mg) / bulb (% of injury)	% of root length growth as compared to control
	Before Expt.	After Expt.						
0	7.45	7.26	23.68 ± 2.4 a	8 - 55	36.85 ± 4.3 (0) a	269.32 ± 32.5 (0) a	28.26 ± 2.9 (0) a	100
25	7.00	7.01	23.21 ± 2.1 a	6 - 56	36.02 ± 3.8 (2.25) a	271.17 ± 27.4 (No damage) a	28.18 ± 2.6 (0.29) a	97.75
50	6.48	6.49	21.47 ± 2.6 a	6 - 51	35.35 ± 4.0 (4.07) a	258.53 ± 24.9 (4.01) a	27.87 ± 2.4 (1.38) a	95.93
75	6.02	6.52	21.50 ± 2.2 a	5 - 52	34.88 ± 3.6 (5.35) a	258.77 ± 28.6 (3.92) a	25.89 ± 2.8 (8.39) a	94.65
100	5.05	5.26	15.76 ± 1.9 b	4 - 41	20.23 ± 1.9 (45.10) b	189.96 ± 20.1 (29.47) b	20.21 ± 2.2 (28.49) b	54.90
150	4.54	4.29	12.14 ± 1.1 b	3 - 18	12.57 ± 1.9 (65.89) c	101.32 ± 12.0 (62.38) c	9.84 ± 1.1 (65.18) c	34.11
200	4.13	4.12	9.33 ± 1.0 c	2 - 13	5.68 ± 0.7 (84.89) d	47.46 ± 5.2 (82.38) d	5.03 ± 0.6 (82.20) d	15.41

*Mean value of roots from 6 bulbs growing in two plastiwares.

Any two means having a common letter are not significantly different at 5%P level.

Table 5. Microscopic effects on dividing root cells and chromosomes of *Allium cepa* after 48h of exposure to aluminium as $AlCl_3.6H_2O$

Al^{3+} Conc. In mg L^{-1}	Solution pH Before Experiment	Number of analysed mitoses	#Mitotic Index ± S.D.	Total Aberrant Mitoses	% of aberrations of analysed mitoses	Tetraploid Cells
0	4.72	192	8.0 ± 0.7 a	1	0.52	-
2.5	4.70	187	8.3 ± 0.6 a	3	1.60	-
5	4.73	192	5.5 ± 0.5 b	7	3.65**	1
10	4.72	101	2.7 ± 0.2 c	6	5.94**	3
15	4.75	76	1.7 ± 0.1 d	8	10.53***	4

Mean value of M.I. of roots from 6 bulbs.

Any two means having a common letter are not significantly different at 5%P level.

* Significant at 5%P level ** Significant at 1%P level *** Significant at 0.1%P level

Table 6. Microscopic effects on dividing root cells and chromosomes of *Allium cepa* after 48h of exposure to aluminium as $AlCl_3.6H_2O$ along with different doses of calcium as $CaCO_3$.

Conc. in mg L $^{-1}$		Solution pH Before Experiment	Number of Analysed mitoses	#Mitotic Index ± S.D.	Total Aberrant Mitoses	% of aberrations of analysed mitoses	Tetraploid Cells
Control		4.67	198	8.3 ± 0.5 a	2	1.01	-
Al^{3+} 10 mg L $^{-1}$		4.55	114	3.0 ± 0.2 b	8	7.01***	4
Al^{3+} 10 mgL^{-1} +	Ca 50 mgL^{-1}	6.09	149	3.2 ± 0.3 b	7	4.70*	2
	Ca 100 mgL^{-1}	6.68	165	4.9 ± 0.6 c	4	2.42	2
	Ca 150 mgL^{-1}	7.14	192	6.8 ± 0.6 ac	4	2.08	2
	Ca 200 mgL^{-1}	7.43	189	8.4 ± 0.7 a	1	0.53	-

Mean value of M.I. of roots from 6 bulbs.

Any two means having a common letter are not significantly different at 5%P level.

* Significant at 5%P level ** Significant at 1%P level *** Significant at 0.1%P level

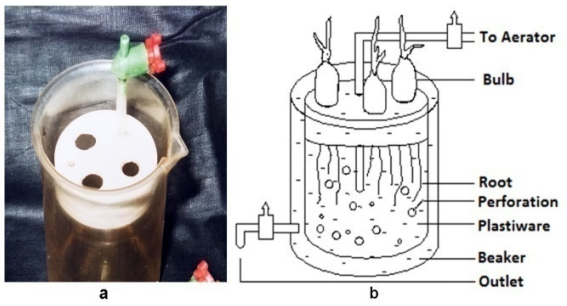

Figure 1. New Set-up (Self-devised) of *Allium* test: a. Original set-up; b. Diagrammatic

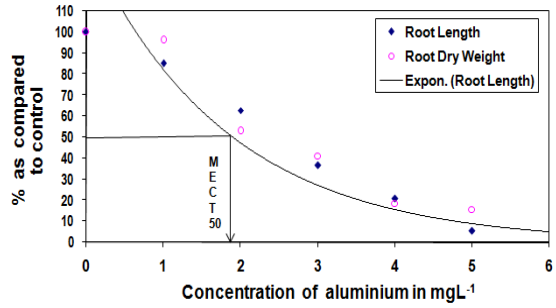

Figure 2. Rhizotoxicity of aluminium in *Allium cepa* after 72h of exposure (Conventional Method)

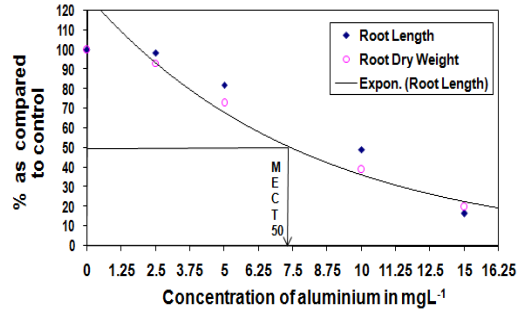

Figure 3. Rhizotoxicity of aluminium in *Allium cepa* after 72h of exposure (New Set-up)

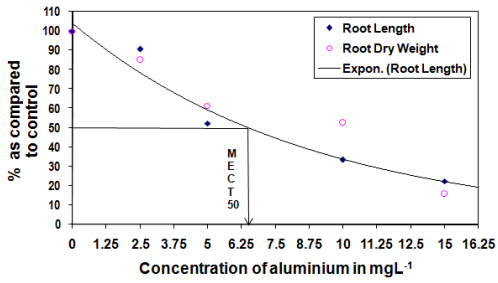

Figure 4. Rhizotoxicity of aluminium in *Allium cepa* after 72h of exposure in dilute nutrient medium (New Set-up)

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RESEARCH ARTICLE

Effect of Carbohydrate Nutrient as Rice Waste on Performance of Earthworm: *Eisenia foetida*

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ABSTRACT

Vermiculture technology is emerging as an environmentally sustainable, economically viable and socially acceptable technology throughout the globe. The study of vermiculture was designed to evaluate the growth, reproduction, advantages of earthworm on different locally available organic manure and it's service to mankind. There are infinity advantages of earth worm that act in the soil as aerators, grinders, degraders and biological stimulators. The growth and reproduction of earthworm species measure by different parameters such as growth by length, body weight, production of cocoons as indicator of population. It also provided to find out the favorable condition for earthworm to grow by measuring the parameters such as temperature, humidity, electro-conductivity, pH. The present study was established to estimate the growth rate of *Eisenia foetida* with respect to various proportion such as 20%, 40%, 60%, 80% and 100% of cooked rice waste. The use of earthworm as "waste manager" for efficient composting of food waste and as "soil managers" for fertility improvement and enhance agriculture production was determined through changing physico-chemical parameters, survivability and population growth of *Eisenia foetida* in each proportion was estimated.

Keywords: Vermiculture, earthworm, *Eisenia foetida*, Rice waste (Rw)

INTRODUCTION

The current scenario of increase in population possess increase in the quantity and type of urban and rural waste. The production source of wastage are due to urbanization and industrialization towards fulfillment of demand of

human kind. All cities and towns of India like other developing countries also suffer with solid waste management (Kaur and Joshi, 2002). About 25 million tons of municipal solid waste, 320 million ton of agricultural waste. 210 million tons of cattle manure and the food wastes were generated as a major global problem. According to the Food and Agriculture Organization (FAO) of UN approximately one third of food is produced from the human need. Nearly 40% of food wastage was generated from India evaluated by the UN. Out of various organic wastage the cooked food are released from major sources like restaurant, celebration events, hostels, hotels, holly places and houses etc. If these organic waste not treated in-time and effective manners these organic waste causes environmental pollution and badly produce fouling smell that harmful for human health. These type of solid waste can be converted into useful product such as organic manure needs for the plant growth are developed. Various biological management for organic wastes instead of disposal to land fill site, open dumping or any other environmentally risky waste management but vermicomposting is the most providential method for recycling (Sangwan et al.,2002, Aalok et al., 2008, Adhikary et al., 2012)

Vermicomposting is one of the biological treatment of solid waste converts into useful organic manure as humus. It provide ruminative organic manure, nontoxic and odour free. The worms feed on organic materials breakdown into the simpler form in the body and excrete it as casting or vermicompost. This casting helps in improving the quality of soil drainage and aeration. A scientific method of breeding and multiplication of earthworm applying in vermicomposting pits with control condition is named as vermiculture. The aim of this is creating such improved conditions artificially where the earthworm can multiply shortest period of time and space. Vermiculture approximately source of biofertilizer (Bhawalker,1989) and it is clearing of environment with cost effective management technology (Sultan Ismail, 1993 and Gunnathilagaraj, 1994). Earthworm over 600million year of experienced as 'Ecosystem engineers' by vermiculture scientist all over the world knew about the role of earthworm as 'waste manager' and 'fertility improves'. *Eisenia foetida* are the best species used for vermicomposting and vermiculture are both" protective" and "productive "for environmental and society.

About *Eisenia foetida*

Eisenia foetida also known as red worm, tiger worm, brandling worm etc. they recharged ground water and maintain soil temperature and moisture also increase root volume and bacterial activity. It act as a grinder, crusher, chemical degrader and biological stimulator, they also known as silent machines or biological indicator of soil fertility that have been performing incredible function of ploughing and fertilizing soil.

Systematic position

Phylum -Annelida
Class-Clitellata
Order-Haplotaxida
Family-Lumbricidae
Genus-Eisenia
Species- *Eisenia foetida*

The prime objective of the present study was to produce population growth of *E. foetida* through vermiculture process by using different proportion of nutrition carbohydrate as cooked rice waste which later can convert to organic manure for a sustainable plant growth. The production of vermicompost by recycling of rice waste was due to increase in population that indicated the survivability in the given condition and facilitated the consumption of rice waste into organic fertilizer which assessed through different physico-chemical parameters.

MATERIALS AND METHOD

The present experiment study on vermiculture was carried out approximately about 60-70days from January to March inside the campus of CUTM located at Jatni, Bhubaneswar, Odisha, India.

COLLECTION OF SAMPLES

- Rice waste was used as nutrients for vermiculture collected from the canteen of girls' hostel, CUTM campus.
- Fresh cattle dung was collected in a plastic bags from dairy farm and soil was collected from the garden area of CUTM campus.
- The exotic healthy species *Eisenia foetida* was used for the purpose of vermiculture were collected from the vermiculture unit of the campus and kept in a separate controlled environment in Department of Zoology before experiment.
- Six rectangular plastic pot with 28cm in length,17 cm in breadth and 11 cm in height from the bottom to the top were collected and used in vermiculture.

PREPARATION FOR VERMICULTURE

The collected cow dung and soil were allowed to dry in sunlight for a week spreading over plastic sheets. The required cow dung and soil were completely dried and chopped into smaller particles. The air dry materials were sieved by a sieve of 2.6 mm and collected in plastic bags. Then after the material were brought into weighing site for the measurement of different proportion of 20%,40%,60%,80% and 100% of rice OM whereas equal amount of soil + cow dung (OM) together taken as 100%.

PREPAREATION OF VERMICOMPOSTING

The experiment was designed with the following proportion by taking 6 rectangular pots labelled as
Control = (OM) (1:1)
Set -1—(OM+RW)(4:1)
Set-2---(OM+ RW)(3:2)
Set-3 ---(OM+RW)(2:3)
Set-4---(OM+ RW) (1:4)

All the proportional materials were allowed to mix well and applied to form vermi-bed by layering the mixture with addition of slurry in each labeled pots and kept in shaded area by sprinkling of water daily twice upto 10-15 days to maintain moisture. The earthworm *E.foetida* were measured with their length, perimeter, and diameter ad allowed to introduce 3 in number into vermibed of each container after pre-composting. After allotted into all different pots by adding slurry of cow dung at top of the bed including rice waste for maintaining nutrition of earthworm. Water was sprinkled in regular interval to all pots for maintaining moisture content and also room temperature. Monitoring of the process from every aspects was carried out up to 60-70 days with obtaining vermicompost with respect to the proportion mixture that harvested .The physical parameters such as moisture content of mixture, pH, electro-conductivity, survival ,growth and population of earthworm were estimated before and after vermiculture.

RESULT AND DISCUSSION

The present study worked out with various proportions of cooked rice wastes was subsequently produced with organic manure by using vermicomposting for production of enriched organic manure. The physico-chemical parameters like moisture content, pH and electro-conductivity were easily controlled and indicated the progress of vermicomposting. Graphical analysis of pH, electro-conductivity and moisture content were studied in Table 1. and

Figs. (1.,2,3&4) . Physico–chemical variables in each proportion from the present work was found varies in each sets. The output with better result was observed in both Set-1 and Set-2 as compared to other pots in respect to all physico-chemical parameters. Rapid consumption of organic rice wastes by earthworm crushed into finer particle through the muscular grinding organ gizzard and released dark black granular vermicast (Lakshmi Prabha et al 2014). The earthworms were obtained their nutrition from microorganism during vermicomposting that grow upon the wastes. During pre-composting the microbes were produced and undergoes a process of fermentation which converted the mixtures into acidic medium then later reached to slightly acidic (Jadhav et al, 2013). The pH was maintained within 6.5 to 7.6 i.e. in vermicomposting pots from control to experiment. The pH in all pots were slightly acidic except Set-3 shown alkalinity while the experimental was acidic (Table-1 & Fig.-2) might have caused by the microbial nitrification process (Eklind and Kirchmann ,2000, Singh,et al 2005).

The moisture content was maintained by sprinkling water into all pots at 65%-75%,where it was more in control (Table1. & Fig.-3). The temperature was maintained below 35^0C for survival earthworms (Taiwo and Oso, 2004). The temperature in Set-4 and experimental of present study was shown more than 35^0C causes death of earthworm (Table1. & Fig.4) for overheating due to fermentation. From preliminary stage of vermicomposting to till completion the earthworms *E. foetida* survived in almost all pots except Set-4 where it was rare and nil at experimental (Table2. & Fig.-5).Soil acidification (Ma et al., 1990), decreased the soil aggregate stability (Estevez et al., 1996) decrease in soil respiration (Sharma 2003), pollution of underground water and decrease in earthworm populations (Edwards and Bohlen 1996). The Electro-conductivity (EC) was increased in initial stage due to release of different mineral salts available in mixture during the process of pre-composting and later the available salts were converted into insoluble salts which may be the reason for the reduction of EC as the vermicomposting process (Nisha Jain,2016). From this present study it was observed that the growth of earthworm was suitable with respect to all physico-chemical parameters available such as in Set-1, Set-2 in maximum rate, Set-3 was showing with moderate value and in Set-4, experimental achieved with minimum range in respect to standard proportion taken as control. The survival and growth of earthworm *E. foetida* was led to produce juveniles subjected to population growth which indicated the faster production of vermicompost in Set-1and Set-2 contained less amount of rice waste. In others such as Set-3,Set-4 and experimental less or no vermicompost was produced due to low or not at all production of juveniles during vermiculture. So the present result explained that the population production is only possible in suitable proportion of organic waste lead to produce vermicompost i.e. more population faster vermicomposting.

CONCLUSION

The carbohydrate content rice waste from the present study was observed that the waste along with addition of soil and cow dung degraded slowly during vermicomposting technique. It can be able to convert in to sustainable agriculture application by the action of earthworm along with microbes play an important role in producing organic rich manure as vermicompost. The population of earthworm was produced the enriched and product of vermicasts with high carbon nitrogen profile as confirmed through different parameters. It help to reduce the volume of rice waste additionally to produce eco-friendly and cost effective manure for agricultural field.

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Tabulation.1 Measurement of physio-chemical parameters of various observations

Sl. No	Obser vation	Sample In prop-ortion	Electroconductivity in S/m			P^H			Moisture content		
			Initial	Final	Differ-ence	Initial	Final	Differ ence	Before vermicult ure	After vermicult ure	Differ-ence
1	Control-100%	OM (100%)	0.275	0.357	0.082	6.27	6.25	0.02	1.2728	1.2828	0.0152
2	Set-1	Rw+OM (1:4)	1.525	1.496	0.029	5.55	5.50	0.o5	0.9411	0.9499	0.0088
3	Set-2	Rw+OM (2:3)	1.983	2.20	0.217	7.23	7.20	0.03	0.3386	0.4486	0.11
4	Set-3	Rw+OM (3:2)	1.323	0.993	0.33	5.o5	5.30	_0.25	0.8356	0.9666	0.131
5	Set-4	Rw+OM (4:1)	1.096	1.050	0.046	6.o4	6.00	0.04	0.7796	0.8697	0.0901
6	Expt.(FA)	Rw (100 %)	1.010	0.530	0.48	6.40	6.37	0.03	0.3196	0.4094	0.0898

Tabulation.2 Survival number of earthworm in each proportion during vermicomposting

SL. No	Proportional setup	Initial number of earthworm	Survival of earthworm in 1st 15days	Survival in next 2nd 15days/after 1 month	Survival in 3rd 15 days	Survival in 4th 15days
1.	Control	3	4	7	11	21
2.	Set-1	3	3	4	8	16
3.	Set-2	3	4	6	10	13
4.	Set-3	3	3	5	7	9
5.	Set-4	3	2	1	0	0
6.	Experimental	3	2	0	0	0

Tabulation. 3 Growth of earthworms before and after vermicomposting in various proportion.

	INITIAL		FINAL	
	Length(cm)	**Diameter(cm)**	**Length(cm)**	**Diameter(cm)**
Control	11.5	1.3	12	11.4
	10.7	1	11	1.2
	10.9	1.1	11.2	1.3
Set-1	7.5	1.1	7.8	1.2
	7.4	1.3	7.6	1.4
	6.9	0.9	7.3	1
Set-2	10.2	1.3	10.3	1.4
	10.3	1.7	10.6	1.8
	10.2	1.8	10.5	1.9
Set-3	9.9	2	10.3	1
	9.8	1.5	10.1	1.6
	8.5	1	8.8	1.2
Set-4	7.7	0.8	7.9	1
	7.8	0.7	8.1	1.1
	6.7	0.5	7	0.8
Experimental	5.5	0.7	5.7	0.9
	5.7	0.8	5.9	0.9
	4.9	0.3	5.4	0.5

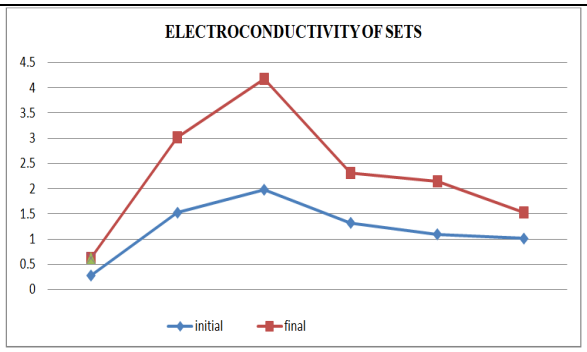

Fig.1 Graphical presentation of electro-conductivity in each set

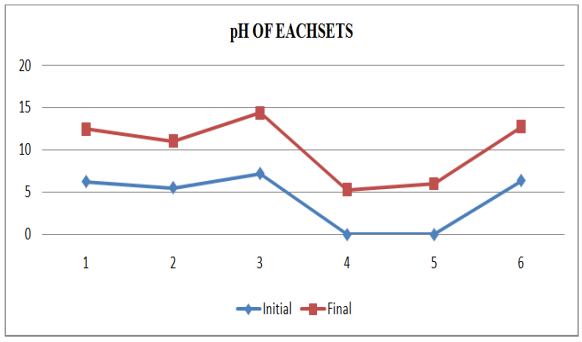

Fig.2 Graphical presentation of pH in each set

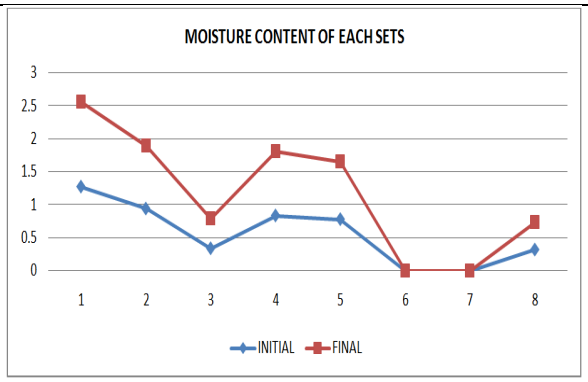

Fig.3 Graphical presentation of moisture content in each set

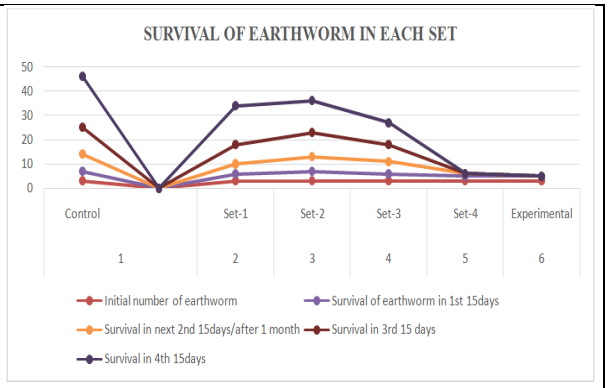

Fig.4 Graphical presentation of earthworm survivability

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RESEARCH ARTICLE

Comparative Evaluation of Anti-Inflammatory Potential of Ethanolic Extract of Bamboo Leaf with Ibuprofen-An *In vitro* Analysis

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ABSTRACT

Endodontics has always been indebted to pharmaceutical sciences to provide it with necessary analgesics and anti-inflammatory agents. More specifically, there has always been a need for anti-inflammatory phytotherapeutic agents as the commercially available synthetic anti-inflammatory drugs have their own limitations due to undesirable side effects. Hence, novel potent analgesic and anti-inflammatory drugs without considerable side effects from the natural sources are under evaluation. *Bambusa vulgaris* is a plant found throughout India and in most of the Asian countries. It has been shown to have variety of medicinal properties. In the present study, we have shown that potential anti inflammatory activity of *Bambusa vulgaris* and compared with standard drug. Aim: To evaluate the *in vitro* anti inflammatory potential of different parts of *B.vulgaris* ethanolic extract and to compare the anti inflammatory activity with standard drug ibuprofen. Methodology: The ethanolic extraction of *Bambusa vulgaris* leaves was done as per the standard method. Different concentrations (25-250µg/ml) of the extracts were used for anti-inflammatory activity by inhibition of albumin denaturation. All samples were analyzed in triplicate. The results were statistically analyzed. Results: The ethanolic extract of *Bambusa vulgaris* leaf have shown to have anti inflammatory activity in a dose-dependent manner. However, the anti-inflammatory potential of *Bambusa vulgaris* is comparable with that of the standard drug ibuprofen.

Conclusion: It is concluded from the present findings that *B.vulgaris* possesses anti-inflammatory properties which could be due to presence of active constituents present in the plant extracts. Hence, *Bambusa vulgaris* may serve as one of the anti-inflammatory herbal drugs for Endodontic infection-induced inflammation and related to dental diseases. Further studies on the identification of the active principles present in the leaf of *Bambusa vulgaris* is needed to find its potential.

Keywords: *Bambusa vulgaris,* Anti-inflammatory effect, ethanolic extract, protein denaturation, HRBC membrane stabilization.

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluids and blood cells. Though it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain, or aggravate many diseases(1)[Sosa et al]. Endodontics field needs the use of necessary analgesics and anti-inflammatory agents for the success of treatment. More specifically, there has always been a need for anti-inflammatory agents, because of the undesirable side effects of the wide range of drugs used in root canal treatment. So to be used in endodontics, the drug should be delivered both systemically and topically to reduce the inflammation, without causing side effects. Hence, the development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary. Bamboo is a potential source of bioactive substances as well as a useful building material. Among the different parts of the bamboo plant, the roots and leaves have been used for food and medicine. Some studies of bamboo leaves extract have identified its biological effects, which include antioxidant, anticancer, and antibiotic activity (2)[Zhang et al]. The leaves of *Bambusa vulgaris* have been used in Indian folk medicine to treat various inflammatory conditions. Many ingredients have been isolated from the leaves, including polyphenols (flavones, phenolic acids, glycosides, and coumarin lactones), anthraquinones, and amino acids (3-5)[Jiao et al, Hasegawa et al and Lu et al].

There are studies on various beneficial effects like anti oxidant property, anti microbial property, astringent property of *Bambusa vulgaris* extract. But the anti-inflammatory property of ethanolic extract of *Bambusa vulgaris* leaves is not widely studied in literature. Hence, this study evaluates the Invitro anti-inflammatory potential of ethanolic extract of bamboo leaf and compares the anti-inflammatory action with standard drug ibuprofen.

MATERIALS AND METHODS

Preparation of extract

The dried bamboo leaf powder was extracted with solvents in 1:5 ratio using soxhlet apparatus for 6-8 hours. From the extract the supernatant was filtered over Whatman No. 1 filter paper. The filtered extract was then subjected to dryness under reduced pressure at 37 ºC (not exceeding 40 ºC), and stored at 4ºC. All the extracts were stored in a dessicator for further evaluation.

In vitro anti-inflammatory Assay

HRBC membrane stabilization method

The anti-inflammatory activity of plant extract was assessed by in vitro HRBC membrane stabilization method. Fresh blood was collected and mixed with equal volume of sterilized Alsever solution and stored at 4ºC and used within 5 hrs. Alsever solution contains 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride. Saline at two different concentrations were prepared (isosaline 0.85% and hypo saline 0.25%). Add 19 ml 0f 0.5 M sodium

dihydrogen phosphate solution to 81 ml of 0.15 M sodium hydrogen phosphate solution. Check the pH and adjust with monobasic or dibasic solutions as required. Store at room temperature.

RBC suspension: The blood samples were centrifuged at 3000 rpm at room temperature for 10 minutes and the packed cells obtained were washed with isosaline (pH 7.2) 3 times and 10% (v/v) suspension was made with isosaline. The assay mixture contained different concentration of extract (25-250µg/ml) and for standard Ibuprofen (1µg/ml), 1 ml of phosphate buffer (0.15 M, pH 7.4), 2 ml of hypo saline and 0.5 ml of 10% RBC suspension. In another tube 2ml of distilled water was taken and this served as the control. All the tubes were incubated at 37ºC for 30 min. Then it was centrifuged and the hemoglobin content in the supernatant was estimated using UV-spectrophotometer at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula,

Percentage protection = 100 - OD of Test /OD of Control x100

Albumin Denaturation

The reaction mixture was consisting of test extract and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCL. The sample extracts were incubated at 37ºC for 20 min and then heated to 51ºC for 20 min, after cooling the samples the turbidity was measured at 660nm(UV Visible Spectrophotometer Model 371, Elico India Ltd). The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:
Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

Statistical Analysis

The experiments were carried out in triplicates; the results were represented as Mean ± SEM. Statistical differences were determined by one-way analysis of variance (ANOVA) and post hoc comparison test. P-values <0.05 were considered statistically significant. Data were analyzed using the SPSS 22.0 package (Chicago, IL, USA).

RESULTS

Figure 1 and Table 1 represent the effect of different concentration of *B.vulgaris* ethanolic extract on HRBC membrane stabilization. The *In vitro* anti inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane (6,7) and its stabilization implies that the extract may well stabilize lysosomal membranes. The protection of RBC membrane by the ethanolic leaf extract at 250 µg/ml concentration showed 62% respectively which could comparable with the commercially available synthetic anti inflammatory drug Ibuprofen. Figure 2 and Table 2 represent the effect of different concentration of *B.vulgaris* ethanolic extract on inhibition of albumin denaturation. Protein denaturation is a well established cause of inflammation. As a part of the investigation on anti-inflammatory activity, ability of different concentration of *B.vulgaris* ethanolic extracts showed differential inhibitory activity (Table 2 and Figure 2). The inhibitory activity of leaf extract at 250 µg/ml concentration showed 71% respectively which could comparable with the commercially available synthetic anti inflammatory drug Ibuprofen. This study shows significant difference in HRBC membrane stabilization and inhibition of albumin denaturation when compared with negative control. But there is no significant difference when compared with positive control (ibuprofen).

DISCUSSION

Prolonged inflammation is the major reason for failure in endodontics. In order to test such activities, laboratory animal models are necessary. Before attempting the newer herbal products on animal models, the first attempt was done in In vitro laboratory tests. The presence of various phytochemicals in these plant products gives a promising

area in anti-inflammatory studies. However, scanning these phytochemicals for all possible biological activity is a huge task and hence should be subjected to initial screening before subjecting them to expensive testing models. Bamboo leaves have been widely used in traditional Chinese medicine and many other Asian countries for treating fever and residential detoxification for over 1000 years. Many biological effects of bamboo leaf extract have been reported, such as antitumor (8), antibacterial (9), and antioxidant (10) activity, and it can be used as an intermediate in the synthesis of pharmaceuticals and food additives. Although the most abundant components are hesperidin and naringin, the major functional components of bamboo leaf extract are flavones C-glycosides such as orientin, isoorientin, and vitexin, which have been used as chemical markers for the determination of flavanoids in commercial bamboo leaf products (10-12). The pharmacological activity of hesperidin and naringin suppresses the inflammatory response when compared to other flavanoids (13, 14).

The HRBC membrane stabilization has been used as a method to study the Invitro anti inflammatory activity, because the erythrocyte membrane is analogous to the lysosomal membrane (6, 7) and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (15).

Protein denaturation is the most common cause of prolonged inflammation. Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Mizushima and Kobayashi (1968) have shown that when these phytochemicals inhibit protein denaturation and they have anti-inflammatory activity (16). Sakat *et al*., (2010) have also slightly modified the technique to screen the anti-inflammatory activity *in vitro* (17). The methanolic extract of *E.axillare* has been studied successfully (18). The relation between *in vitro* protein denaturation and *in vivo* anti-inflammatory action has been seen in *Mikania scandens* (19). Hence in this study, as a part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied.

The phytochemicals analysis of the extract revealed that it contains flavanoids, carbohydrates, glycosides, proteins, and alkaloids. Of these, flavanoids and alkaloids are well known for their ability to inhibit pain and inflammation. Flavanoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediators of inflammation (20). Finally, we van correlate that this study's results support the traditional use of this plant in some inflammatory and painful conditions and confirm the presence of active chemical compounds related to these activities. Previous studies have shown that bamboo leaves extracts has the ability of either inhibiting free radical formation or itself be a free radical scavenger (21). The above results highlight the ability of different bamboo leaves extract to scavenge DPPH free radicals by their proton-donating ability. Consistent with previous research, the result obtained here, indicates that anti-oxidant activity of the various extract of *B. vulgaris* and *B. nutans* increases with total polyphenolic content. And also a study by Samy et al proved that *Bambusa vulgaris* is a potent antimicrobial agent (22).

In current study, it was seen that as the concentration of extract increased there was a steady rise in anti-inflammatory activity in both the analysis. In relevance to dose dependent protein protective activity, the change in concentration of extract showed proportional increase in activity. This was almost linear and hence be attributed only to increase in phytochemicals content. In certain cases, small change in dose can produce a large change in activity. At times, phytochemicals can also exhibit therapeutic window. In this study, such phenomena were not observed in the selected concentrations. This also means that the concentration almost achieved saturation as it

reached higher levels. This suggests that at dose cannot be indefinitely increased and maximum protective activity will be seen at around 250μg/ml concentrations. Though direct correlation of this value to *in vivo* studies cannot be found, it stands as a guideline for proper dosing.

CONCLUSION

Our present study concludes that *Bambusa vulgaris* possess anti-inflammatory properties which could be due to presence of active constituents present in the plant extracts. Hence, *Bambusa vulgaris* may serve as one of the anti inflammatory herbal drugs for Endodontic infection-induced inflammation and related to dental diseases. Further studies on the identification of the active principles present in the leaf extract are warranted to ascertain its potentials. In future, oral bioavailability and mechanism of action should be elaborated and the drug should be used in pre-clinical lab animal models.

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Table 1: HRBC Membrane stabilization

Sample	Concentration (µg/ml)	Absorbance at 560nm	% Protection
Negative control	-	0.402 ± 0.03	-
Bamboo leaf Extract	25	0.354 ± 0.03	11.9 ± 1.1*
	50	0.296 ± 0.02	26.3 ± 1.5*
	100	0.214 ± 0.01	46.7 ± 2.4*
	200	0.196 ± 0.01	51.2 ± 3.5*
	250	0.154 ± 0.01	61.6 ± 4.9*
Ibuprofen (1 µg/ml)		0.132 ± 0.01	67.1 ± 5.2*
IC_{50} value	-	-	107.6µg/ml

Results were expressed as Mean ± SEM of n=3. *p<0.001 statistically significant as compared with Negative control.

Table 2: Inhibition of Albumin Denaturation

Sample	Concentration (µg/ml)	Absorbance at 660nm	% Inhibition
Negative control	-	0.395 ± 0.03	-
Bamboo leaf Extract	25	0.304 ± 0.02	23.0 ± 0.98*
	50	0.268 ± 0.02	32.1 ± 0.85*
	100	0.189 ± 0.01	52.1 ± 0.63*
	200	0.135 ± 0.01	65.82 ± 0.57*
	250	0.115 ± 0.01	70.88 ± 0.43*
Ibuprofen (1 µg/ml)		0.101 ± 0.01	74.4 ± 0.12*
IC_{50} value	-	-	77.88µg/ml

Results were expressed as Mean ± SEM of n=3. *p<0.001 statistically significant as compared with Negative control.

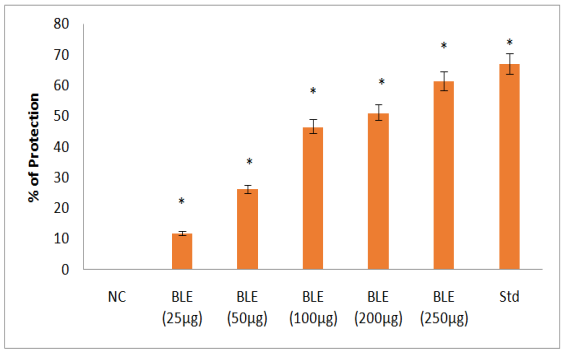

Figure 1: This graph depicts the percentage of RBC cell membrane protection. Results were expressed as Mean ± SEM of n=3. *p<0.001 statistically significant as compared with Negative control.

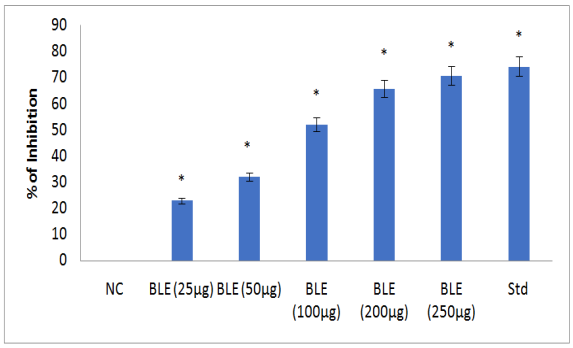

Figure 2: This graph depicts the percentage of albumin denaturation inhibition. Results were expressed as Mean ± SEM of n=3. *p<0.001 statistically significant as compared with Negative control.

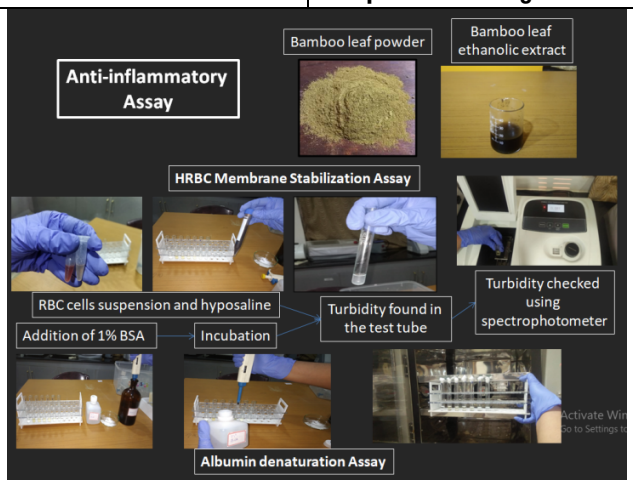

Figure 3. Anti-inflammatory Assay

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RESEARCH ARTICLE

Enzymatic Saccharification of Rice Straw and Fermentative Production and Detection of Value Added Products

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ABSTRACT

Enzymatic hydrolysis is essential to complete cellulase degradation. Action of cellulase leading to the production of glucose would make the production of bioethanol from lignocellulosic material more profitable. In the present study, saccharification of agro-residues like rice straw was carried out by crude enzyme. Enzymes synthesized on rice straw medium released sugars which latterly identified as glucose and xylose by TLC, HPTLC as well as FTIR. Sugar (67 mg %) was added in the fermentation broth and incubated with *Saccharomyces cerevisiae* for 48 hrs and analyzed for ethanol concentration after distillation by dichromate method. 3.9 g/l ethanol obtained after optimization of various parameters affecting. Detection of ethanol also confirmed by Gas Chromatography (GC). Obtained sugars also successfully utilized and optimized for production of sorbitol (Sugar Alcohol) by *Zymomonas mobilis (MTCC2428)* confirmed by TLC as well as HPTLC analysis. Similarly experiment for production of Glycerol was carried out which was further detected by Gas Chromatography. Experiment was materialized for purification of ethanol by redistillation as well as by chemical dehydration to utilize for blending with gasoline to run SI engine to check efficiency as biofuel which successfully done upto 40% blending.

Keywords: Rice, population, productivity, nutrient management, INM

INTRODUCTION

Rice straw is one of the abundant lignocellulosic waste materials in the world. It is annually produced in large quantity reaching about 731 million tons distributed in Africa (20.9 million tons), Asia (667.6 million tons), Europe (3.9 million tons) and America (37.2 million tons). This amount of rice straw can potentially produce 205 billion liters bioethanol per year, which is the largest amount from a single biomass feedstock. Most reports on ligno-cellulosic ethanol production involve acid hydrolysis followed by enzymatic saccharification. The enzymatic saccharification step is cost-prohibitive because of the high cost of the enzymes. In India, like other countries around the world, there was a considerable shortage of oil and sharp rises in crude oil prices in the 1970s, prompting interest in fuel ethanol.

In India too, plans have been developed to use ethanol-gasoline blends and even ethanol-diesel oil blends. Based on recommendations by a committee for the development of alternative fuels for motor vehicles, trials were carried out in New Delhi in 1991. The test fleet included 93 vehicles and comprised cars, vans and jeeps. The fuels used were blends of 5 % and 10 % ethanol in gasoline (Archana and Satyanarayana, 1997).

MATERIALS AND METHODS

Enzyme Extraction and purification

After incubation, The crude enzyme from each flask was extracted by adding 60 mL of 50mM sodium citrate buffer (pH 5.3), and mix thoroughly and put the flask on orbital shaker at 150 rpm for 1 hour. After 1 hour whole content was filtered through a wet cheese cloth by squeezing. The extract thus obtained was centrifuged at 8000 rpm for 15 min at 4^{o}C. The volume of supernatant and pH was measured and it was used to determine enzyme activity. Addition of solid ammonium sulphate to the enzyme solution with constant stirring at 10°C till achieves 0-40% saturation. After centrifugation at 8000 rpm for 20 min separated the supernatant and precipitates. The precipitates were dissolved in small amount of 0.05M sodium citrate buffer pH-5.3 and the enzyme activity and total protein content was measured from both precipitates and supernatant. After that the supernatant was subsequently adjusted at 40-70 % saturation with addition of ammonium sulphate and centrifudge it again. Precipitates were dissolved in small amount of buffer. Xylanase activity and protein estimation was carried out using both supernatant and precipitates. After ammonium sulphate fractionation, the enzyme solution was dialysis for about 18-24 hours at 10 °C against 0.05 M sodium citrate buffer pH-5.3. Three intermittent changes of buffer were done. Xylanase activity and protein estimation was carried out on the dialyzed sample.

Enzymatic hydrolysis

Alkali Pretreated and untreated rice straw and banana stem, in different 250mL of Erlenmeyer flask, mixed with extracted enzyme having 1.2 U/mL activity and purified enzyme having 1.2 U/mL activity in each flask. The total volume of solution is adjusted to 50mL by addition of 50mM sodium citrate buffer of pH 5.3. This Enzymatic solubilization was performed in an orbital shaker at 50^{0}C, 150 rpm for 24 h. The content of reducing sugar solubilized into hydrolysate was determined by DNSA method at every 2 hours.

Inoculation Preparationfor ethanol production

For the production of ethanol *Saccharomyces cerevisiae* culture was used. For the preparation one isolated colony was taken from the slant and inoculated into GYE broth after 48hrs incubation at 37°C at 150 rpm. After incubation cell density was measured at 600nm using distilled water as a blank by Double beam U.V/Visible Spectrophotometer Elico SL 164. The optical density of suspension was adjusted to 1.0 O.D (1 x 10^{6} cell/ml). For enzymatic hydrolysis, prepared the inoculation, the spores on the PDA slant were suspended in 2ml medium (10^{6} spore ml^{-1}) and then pipette into a 250 ml of Erlenmeyer flask containing 50ml of inoculum growth medium and incubated in a shaking bed (200 rpm) at 30°C. After 3 days growth the medium was used as the inoculum for enzyme hydrolysis.

The medium was a 1000 gL^{-1} solution with 10g; Yeast extract, 10g; beef extract, 2g; peptone, 1g; glucose and suitable $FeSO_4.7H_2O$. The initial pH value of the medium was adjusted to pH 7 before being autoclaved at 121°C for 15 min. which was kept at static condition, at 28°C and after 48hrs, 72hrs, 96 hrs, analysis for reducing sugar and alcohol estimation was done.

Zymomonas mobilis MTCC2428

Zymomonas mobilis MTCC2428was obtained from culture collection center Pune. Culture was first revived in broth of specific media mention below. It was streaked on specific medium constitute in (gL^{-1}), contains yeast extract 10g,

Glucose 20g,KH_2PO_4 2.0g, Agar 15g & pH was adjusted at 6.0. Isolated colonies were traced after 48hrs at 30°C temperature. The pure culture was examined for its morphology and colony characteristics.

Production of Ethanol and Sorbitol by *Zymomonas mobilis* by fermentation

For the production of sorbitol and ethanol by *Zymomonas mobilis* culture was used. For the preparation one colony was taken from the slant and inoculated into specific medium broth after 48hrs incubation at 30°C at 150 rpm. After incubation cell density was measured at 600nm using distilled water as a blank by Double beam U.V/Visible Spectrophotometer Elico SL 164. The optical density of suspension was adjusted to 1.0 O.D (1 x 10^6cell/ml).

Ethanol recovery and estimation

Distillation is almost exclusively at the means for ethanol recovery and purification and various designs used to produce the different product grades. For distillation of ethanol 50ml of fermented broth was taken into the distillation flask and heating mental were operated at 78.3ºC for 30min. distilled ethanol were collected at the flask. The ethanol concentrations were checked by dichromate method and Gas chromatography.

RESULTS

Partial purification

Enzyme produced by solid state fermentation of rice straw by fungal isolate isolate***2b*** (*Aspergillus spp*) is further purified by ammonium sulphate fractionation method and then by performing dialysis of obtained fraction 60-70%. During this fractionation our desired protein got precipitated which is further dialyzed against 0.05M citrate buffer having pH-6.0. Following table (4.5.1) shows the efficiency of purification method. This purified enzyme was stored 4°C and further used for its kinetics studies.

Enzymatic hydrolysis

Enzymatic hydrolysis is essential to complete cellulase degradation. Action of cellulase leading to the production of glucose would make the production of bioethanol from lignocellulosic materials more profitable. In the present study, saccharification of agro-residues like rice straw and banana stem was carried out by crude cellulase enzyme and partially purified cellulase enzyme from the fungal isolate 2b (*Aspergillus sp.*). Figure.1 shows the profile of reducing sugars obtained upon enzymatic hydrolysis of rice straw and banana stem with 5% NaOH treatment and untreated substrates. After an initial phase of rapid sugar formation, there was decrease in the rate of hydrolysis. This could be due to enzyme inactivation or depletion of an easily hydrolysable fraction of cellulase in the mixture. From the observed results, it was found that the sugar yield was maximum from the pretreated banana stem as compared to pretreated rice straw. Enzymes synthesized on banana agro-waste medium released more glucose.(Baig et al., 2003).

Detection of Sugar by HPTLC

Sugar is one of the important polyols (sugar alcohol) produced by several fungi and fermentative bacteria. Sugar determine by HPTLC method. Figure shows the different tack in which track -1 contained standard sugar while in track -2 and track-3 contained sample scan in visible scanner. Sample track shows presence of sugar. Graph shows peaks of different concentration of sugar in standard and sample.

Ethanol recovery and detection by Gas Chromatography

Ethanol was recovered from the broth by distillation method was taken for further confirmation by specialized analytical technique Gas chromatography. By the chromatogram obtained from the Gas Chromatography showed the presence of ethanol by comparing the standard result by the graph that showed the similar retention time (3 min) for both standard and the test. So it indicated the presence of ethanol in the fermentation broth. SrilekhaYadav *et al.*

reported batch fermentation of pretreated rice straw hydrolysate with 31 g/L TRS, ethanol of 12 g/L after 36 h incubation with a yield of 0.4 g/g and productivity of 0.33 g/L/h by co-culture of *Saccharomyces cerevisiae* and *Pichiastipitis*

Qualitative analysis of sorbitol

Sorbitol is a polyol with a sweet and refreshing flavor, and can be used as sweetener, moisturizer, texturizer and softener in the food industry. Other uses include vitamin C, sorbose, glycerol propylene, plastics and resin production. Sorbitol can be used in dietetic foods for diabetics because it is not insulin dependent. The sample of culture broth was centrifuged for 20 min at 6000 rpm. The cell-free supernatant was filtered through a 0.2μm membrane filter before analysis.. The sugars and sorbitol were determined by HPTLC (Waters Associates) using a same solvent system and detection of spot can be done by spraying permanganate and then scan with HPTLC scanner (both UV and Visible).

Qualitative analysis of glycerol

Distillation is almost exclusively at the means for Glycerol recovery and purification And various designs used to produce the different product grades. For distillation of Glycerol 50mL of fermented broth were taken into the distillation flask and heating Mental were operated at 280^{0}C for 2 h. distilled Glycerol were collected at the flask. The Glycerol was detected by GAS Chromatography.

Testing of bioethanol blending with Gasoline in SI engine

The countries like Brazil, Sweden and USA have marked their presence in the Bio-Gasoline based alternative auto fuel sector by the invention of the flexible fuel vehicle technologies and application of ethanol gasoline blends as high as E85 to E100 (Demirbas.,2005). The economic and environmental constraints have compelled a country like India to imply a compulsory utilization of just E10 as fuel in the light duty vehicles. While there is no shortage of sugarcane in India, which is one of the prime sources for automotive ethanol fuel, being the 2nd largest producer in the world, India still lacks the technological advancement in terms of engine modifications and material compatibility problems. Ethanol besides being a renewable bio–mass based alternative fuel has other added performance based properties like the higher octane number, inherent oxygen content, and a higher latent heat of vaporisation which allows a higher power to be extracted from the engine both in the modified and unmodified state.

The performance of a SI four stroke engine was investigated by adding a maximum value of 40% ethanol– 60% gasoline blend over pure gasoline. The basic aim of this study was to substitute only up to 50% ethanol in unleaded gasoline in a small engine, with an idea to apply this investigation in engines of smaller size. We observed smooth running of engine as its optimum performance up to 40% ethanol blending with the engine which comparatively better for conventional engines of India. It means we can permit upto 40% blending of ethanol to gasoline for our transport vehicles. That will reduce consumption of gasoline as well as it lower down rate of pollution.

ACKNOWLEDGEMENT

Investigation of SI engine was done at Mechanical Engineering Department of LDRP College of engineering, KSV Uinversity, Gandhinagar.

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Table1 – Kinetics study of partial purified enzyme

Procedure	Total activity(U)	Total protein (mg)	Specific activity (U/mg)	Purification (fold)
Crude enzyme extract	1494	1.205	1239.83	1
Ammonium sulphate precipitation (60-70%)	2160	0.833	2593.41	2.09
Dialysis	1150	0.317	3627	2.92

Table 2 The Comparisons of The Gasoline and Ethanol thermodynamic Properties

Properties	Ethanol	Gasoline
Chemical Formula	C2H5OH	mCnH2n
Boiling Point, deg C @ 1 bar	78	30-225
LHV,MJ/kg Fuel	26.8	44.5
Octane number (Recearch)	111	90-98
Octane number (Motor)	94	82
Stoichometric A/F, mass	8.94	15.04
Flammability limit in air vol%	3.5-26	1.4-7.6
Adiabatic flame temp, Kelvin	2197	2266
Auto-ignition temp, Kelvin	792	753
Molecular weight	46	112
Spacific Gravity at 15.5 ◦ C	0.794	0.71-.75
Latent heat of Vapourisation,kcal/kg	204	70-100
Flame velocity, cm/sec	48	43
Vapour pressure at 58 ◦C bar	0.21	0.8

Table 3 Effect of various ratio of blending on engine performance

Blending ratio	SFC (10 ml)	Airflow
E10	**39.36/39.57**	**1.0**
E20	**37.57/41.32**	**1.0**
E30	**37.79/39.63**	**1.2**
E40	**38.73/40.13**	**0.8**

CR=8, Load=1, Speed=1300 RPM

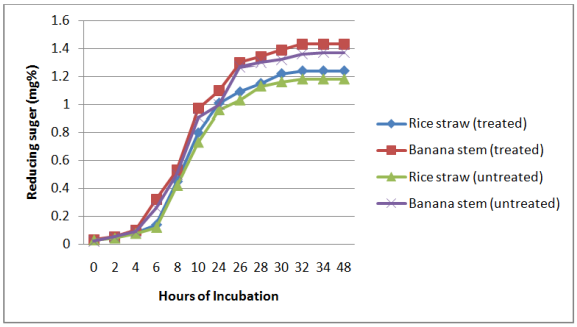

Figure No.1: Substrate hydrolysis of different Agro waste by crude enzyme

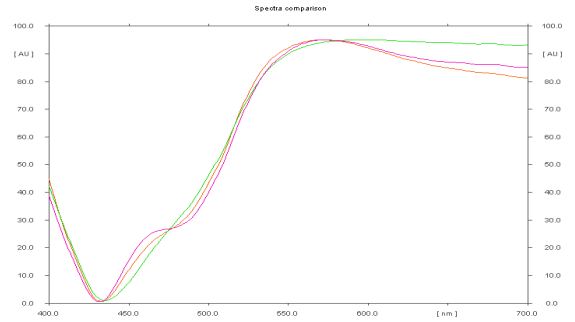

Figure No.2: HPTLC analysis of sacchrified sample

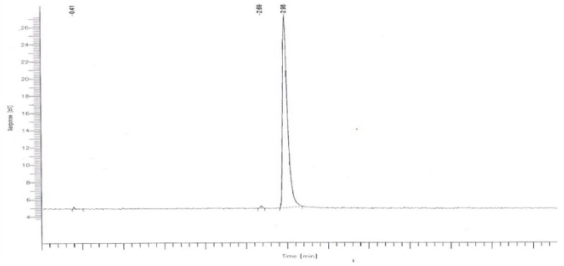

Figure 3 Gas Chromatogram of Recovered sample of produced ethanol

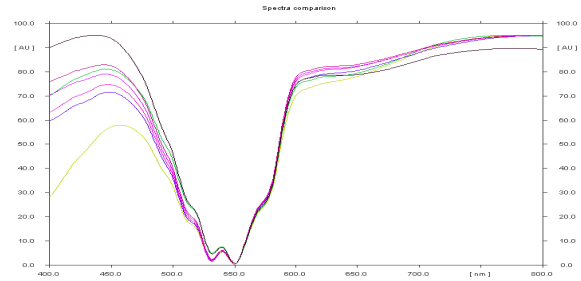

Figure 4 HPTLC analysis of Sorbitol (Standard and Sample)

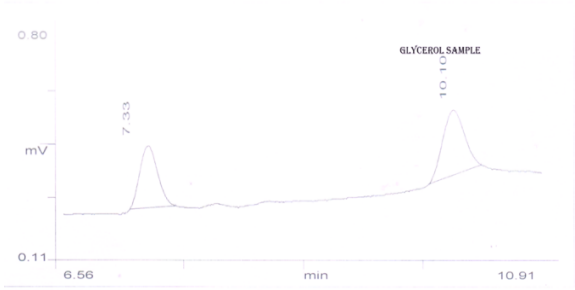

Figure 5. GAS Chromatograph of Glycerol Sample

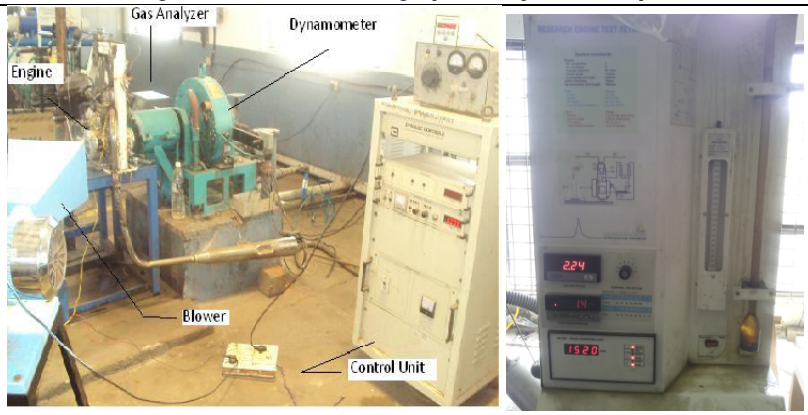

Figure .6 Setup for bioethanol and gasoline blend

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RESEARCH ARTICLE

Screening of Medicinal Plants for Antimicrobial Activity against Plant Pathogens

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ABSTRACT

Medicinal plants serve as a good source of antimicrobial compounds against plant pathogens. About 77 medicinal plants were collected from different parts of Tamil Nadu and screened for the presence of antimicrobial activity against fungal plant pathogens. The plant samples were extracted with three different solvents (methanol, chloroform and petroleum ether). Among the medicinal plants, chloroform extract of *Polygonum minus* was found to be most effective in controlling the plant pathogens. The chloroform extract of *Polygonum minus* was found to reduce the mycelial dry weight of pathogen and spore germination under *in vitro* conditions.

Keywords: Antimicrobial compounds, medicinal plants, *Polygonum minus*

INTRODUCTION

India is rich in plant diversity and more than 7500 plant species are used in traditional health care systems. Emerging trend on exploitation of plant based products for human as well as plant health has raised the demand for medicinal plants nowadays. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. A lot of evidences are available to show the use of medicinal plants to cure human diseases from very long period ago. Due to changing climatic conditions abiotic and biotic stresses are known to affect the plant growth and productivity to a larger extent. Under biotic stress, plant pathogens are the major source which affects the agricultural crops. Use of plant protection chemicals to control these pathogens has created the problems like development of resistance in pathogens and accumulation of chemical residues in agricultural products and environment. Traditionally crude extracts of several plants were used as plant protection chemicals eg., neem products, pungam products, etc., Several plant sources still remains unexplored for the presence of antimicrobial

compounds against plant pathogens. Identifying these antimicrobial compounds and exploiting it for plant protection will serve as a better alternative for plant protection chemicals to some extent. With this in view the present research was conducted to explore the antimicrobial activity of certain medicinal plants against plant pathogens.

MATERIALS AND METHODS

Collection of traditionally important medicinal plants

A detailed survey was conducted at various places of four districts of Tamil Nadu *viz.,* Aliyar forest, Pongalur, Thottampatti, T.N. Palayam, Katturpudur, Kallipalayam, Sivanmalai, Namakkal, Salem, Yercaud and Pollachi for collection of traditionally important medicinal plants. A total of 77 medicinal plants were collected from these places. These plant samples were collected based upon the anecdotal evidence and availability.

Test organisms

Plant pathogens-Fungal pathogens

Alternaria helianthi (Hansf.) Tubaki and Nishih.
Alternaria solani (Ell. and Mart.) Jones and Grout.
Aspergillus flavus Link.
Aspergillus niger Van Tiegham
Colletotrichum musae (Berk. Ef curti.) Arx.
Fusarium oxysporum f.sp. *lycopersici* W.C. Snyder and H. N. Hansen
Macrophomina phaseolina (Tassi.) Goid
Rhizoctonia solani J.G. Khunn.
Sclerotium rolfsii Sacc.

Plant samples

Leaves of the plant samples were shade dried at room temperature (28 ± 2°C) for 3-4 days. The dried samples were ground using a mixer and stored in airtight containers at room temperature in the dark.

Preparation of plant extracts (Geyid *et al*., 2005)

All the dried and powdered leaf samples were extracted by percolation with 80 per cent methanol (polar solvent), chloroform (medium polar solvent) and petroleum ether (least polar solvent) at the rate of 1:5 at room temperature for overnight. The extracts were then filtered with country filter paper and concentrated under vaccum in rotary evaporator to get (as a percentage of powdered plant materials) 6-11 per cent of gummy residue. All the extracts were kept in tightly stoppered bottle in a refrigerator until used for the anti-microbial testing.

Preparation of test samples

Hundred mg of dried plant extracts of each plant sample were dissolved in 1 ml of 100 per cent ethanol and used for the antifungal assay against the plant pathogens.

Preparation of test controls

Negative control

Hundred percent ethanol without the test compound was used as the negative control.

Positive control

The antifungal agent ketaconazole at a concentration of 1 mg/ml were used as the positive control.

Preparation of culture media

The fungi were cultured and maintained in potato dextrose agar medium (PDA). For the bioassay, a loopful of the organism was inoculated into 100 ml of the PD broth. The conical flasks were incubated at a temperature of 37°C for 3-6 days.

Agar well diffusion assay (Iqbal *et al.,* 1998)

The sterilized medium was inoculated with the target pathogen and poured into the petriplates and allowed to solidify. Then each petriplate was divided into four equal quarters using a marker pen. Using a sterile cork borer, wells of 6 mm in diameter were made in each quadrat of the plate containing the media. For each organism, 20µl of the prepared plant sample was loaded in each well using sterilized dropping pipette. Two replications were maintained for each treatment. For each microorganism, the positive control and the negative control (two replications each) were also loaded in a separate well. The plates were incubated for 3-4 days and the observations were taken. The observations were made by measuring the inhibition zone (or halo like area), which indicates the absence of microbial growth around the well. The diameter of inhibition zone (DIZ) was measured and the mean D1Z was calculated.

Certification of the effective medicinal plant sample

The effective medicinal plant sample, obtained after screening studies performed against various plant and human pathogens was identified and certified through Botanical Survey of India (BSI), TNAU, Coimbatore -3, Tamil Nadu.

Botanical description of effective medicinal plant – *Polygonum minus* Huds.

Scientific classification

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Caryophyllales
Family	: Polygonaceae Juss.

Bioefficacy of chloroform extract of *P. minus* against fungal pathogens under *in vitro*

The antifungal activity of chloroform extract of *P. minus* was tested against major fungal pathogens of tomato, maize and groundnut under *in vitro* conditions. Respective media for the pathogens (Appendix – I) were prepared freshly and sterilized and was distributed into separate conical flasks (100 ml per flask). Different concentrations of chloroform extract of *P. minus* (0.50, 1.0, 1.5, 2.0 and 2.5%) were added into 100 ml medium. The medium with chloroform extract of *P. minus* was poured into sterile petriplates and allowed to solidify. The individual fungal mycelial discs of 9 mm diameter was taken from actively growing fungal culture and placed at the centre of each petriplate and incubated at room temperature (28 ± 2°C). A control was also maintained without chloroform extract of *P. minus* for comparison. Observations pertaining to the radial growth (mm) of mycelium were taken when complete mycelial growth covered the control plates. The per cent reduction of mycelial growth was calculated using standard procedures (Pandey *et al*., 1982).

Per cent decrease over control = (Dc-Dt)/Dc x 100

Where, Dc = average diameter of fungal growth in control

Dt = average diameter of fungal growth in treatment

Effect of chloroform extract of *P. minus* on mycelial dry weight of fungal pathogens

Mycelial discs (9 mm) of the pathogens (*A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici*) were inoculated into respective broth (Appendix –I) separately containing chloroform extract of *P. minus* (0.50, 1.0, 1.5, 2.0 and 2.5%).

Conical flask without the extract was maintained as control. The treatments were replicated thrice and incubated for 21 days. The mycelium was harvested through filtration with Whatman No. 42 filter paper. The filter paper containing fungal mycelium was oven dried at 70°C for 24 h and the weight of the dried mycelium was determined (Singh *et al*., 1980).

Effect of chloroform extract of *P. minus* on fungal spore germination

The effect of chloroform extract of *P. minus* (0.50, 1.0, 1.5, 2.0 and 2.5%) on fungal spore germination was tested by Cavity Slide method (Montagomery and Moore, 1938). Spores of the pathogen were transferred to test tubes separately by flooding with sterile water and scrapping the culture with glass rod. The transferred spore suspension was centrifuged at 2000 rpm for 10 minutes to remove mycelial fragments. The spore suspension was adjusted to a concentration of 10^6 per ml using a haemocytometer. One drop of the chloroform extract of *P. minus* was added (0.50, 1.0, 1.5, 2.0 and 2.5%) to the cavity slide and allowed to evaporate. One drop of spore suspension (after thorough shaking) was added to the cavity slides and kept in a moist chamber and incubated at room temperature (28 ± 2°C). Sterile distilled water served as control. Three replications were maintained and percentage of spore germination was recorded after 24 h.

RESULTS

A number of medicinal plants were selected for the study to test their antimicrobial activity against selected plant pathogens. The plants were collected based on the anecdotal evidences. The places from which the plant samples were collected are presented in Plate 1. Maximum number of plant samples was obtained from the Aliyar forest - a place of enriched flora and fauna (Plate 2).

Antimicrobial activity of medicinal plants against plant pathogens

The antimicrobial compounds of the medicinal plants were extracted by using three different solvents *viz*., methanol (polar), chloroform (medium polar) and chloroform (least polar). The results of the studies on antimicrobial activity of medicinal plants against plant pathogens (Table 1a-1c) revealed that the medicinal plant, *P. minus* Huds. belonging to the family Polygonaceae possesses broad spectrum of antimicrobial activity against the selected plant pathogens. The chloroform extract of *P. minus* leaves possessed more activity against *A. solani, A. flavus* (Table 1a), *A. niger* (Table 1b) and *F. oxysporum* f.sp. *lycopersici* (Table 1c) among all the selected pathogenic fungi (Plate 4). The chloroform extract was found to inhibit the pathogens more effectively than the methanol and petroleum ether extracts. The diameter of inhibition zones produced by the chloroform extract of *P. minus* (*A. solani* - 3.3 cm, *A. flavus* - 3.1 cm, *A. niger* - 3.2 cm, *F. oxysporum* f.sp. *lycopersici* - 3.4 cm) were comparable to that of the standard antibiotic ketoconazole (*A. solani*- 3.2 cm, *A. flavus*-3.4 cm, *A. niger*-3.5 cm and *F. oxysporum* f.sp. *lycopersici*-3.4 cm).

Minimum inhibitory concentration of chloroform leaf extract of *P. minus* against plant pathogens

Minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible microbial growth.The minimum inhibitory concentration was evaluated for the chloroform leaf extract of *P. minus* against the selected plant pathogenic fungal cultures. Chloroform leaf extract of *P. minus* showed inhibition at a concentration of 10 mg/ml (dilution 1) against *A. helianthi, A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici* (Table 2). Whereas growth was observed in the other three dilutions/concentrations (dilution 2 –1 mg/ml, dilution 3 - 0.1 mg/ml and dilution 4 - 0.01 mg/ml). Growth was observed in all the four dilutions against *C. musae*, *M. phaseolina, R. solani* and *S. rolfsii*. Ketoconazole (positive control) showed no growth at 10 mg/ml (dilution 1) and 1 mg/ml (dilution 2) concentrations, but growth was observed in the other two dilutions. The cells and solvent control (negative control) showed growth in all the dilutions for all the organisms.

Effect of chloroform extract of *P. minus* on mycelial dry weight of fungal plant pathogens

The mycelial growth of all the tested fungal pathogens was significantly reduced by 2.5 and 2.0 per cent chloroform extract of *P. minus* (Table 3). Two per cent chloroform extract of *P. minus* recorded 96.97, 96.56, 96.96 and 96.39 per cent reduced mycelial growth, whereas 2.5 per cent chloroform extract of *P. minus* recorded 96.98, 96.57, 96.96 and 96.38 per cent reduced mycelial growth in *A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici* repectively when compared to the control. All the treatments with various concentrations of chloroform extract of *P. minus* significantly reduced the mycelial growth of all the tested fungal pathogens more effectively when compared to the control.

Effect of chloroform extract of *P. minus* on spore germination of *A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici*

The results of the effect of chloroform extract of *P. minus* on spore germination of *A. solani. A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici* were presented in Table 4. Among the treatments, chloroform extract of *P. minus* at 2.0 and 2.5 per cent concentration were found to be effective and they inhibited the spores of *A. solani. A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici* by 100 per cent invariably. The higher concentration of chloroform extract of *P. minus i.e.,* 2.5 per cent had the same effect when compared to that of the concentration of 2.0 per cent chloroform extract of *P. minus*. Whereas control recorded 80.12, 83.65, 86.10 and 79.50 per cent spore germination in *A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici* respectively.

DISCUSSION

Medicinal plants are known to contain innumerable biologically active compounds (Perumalsamy *et al*., 1999). Plants may offer a new source of antimicrobial agents for use and they produce a great deal of secondary metabolites, many of them with antifungal activities (Varadarajan *et al*., 2007). Plant diseases are the major biotic constraints to crop growth and causes variety of damage and significant yield loss. Currently, Integrated Disease Management (IDM) concept is increasingly adapted in many plant disease management programmes with a view to protect the environment and to maintain the healthy food chain.

Screening of medicinal plants for their antimicrobial activity

Many of the medicinal plants have been found to possess antimicrobial activity against an array of plant pathogens (Singh *et al*., 2001b; Wang *et al*., 2004; Okigbo and Nmeka, 2005; Siva *et al*., 2008). In the present study, 77 medicinal plants belonging to 40 different families were tested for their antimicrobial activity by agar well diffusion assay against the plant pathogens: *A. helianthi, A. solani, A. flavus, A. niger, C. musae, F. oxysporum* f.sp. *lycopersici, M. phaseolina, R. solani, S. rolfsii.* The selected medicinal plants exhibited varying degrees of antimicrobial activity agianst the selected plant pathogens. The inhibition zones produced by the medicinal plants were compared with the inhibition zones of standard antibiotics (Ketoconazole). Among the plants, *Polygonum minus* exhibited better activity in its chloroform extract against the tested pathogens. The results of the *in vitro* screening supported that the chloroform leaf extract of *Polygonum* sp. was most effective against the selected plant pathogens.

The effective medicinal plant sample *P. minus* was identified and certified as *Polygonum minus* Huds. through Botanical Survey of India, Tamil Nadu, India. Certification was done for the conformation of plant sample at species level (Plate 5). The genus *Polygonum* (Polygonaceae), comprising about 300 species (Wang *et al.,* 2005) is distributed world wide in temperate climate region and is widely found in South America. The chloroform extract of *P. minus* showed significant reduction in mycelial growth and spore germination of the selected fungal plant pathogens – *A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici.* Direct inhibition of the spore germination by medicinal plants was reported by many workers (Patil, 1997; Thirupathi *et al*., 1999). The 2 per cent chloroform leaf extract of *P. minus* inhibited the spore germination of *A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici* by cent per

cent. Amaresh and Nargund (2001) reported that, the plant extract of *Lawsonia inermis* inhibited the uredospore germination by 63.2 per cent at 10 per cent concentration. The *Anacardium occidentale* extracts significatly reduced the growth of *A. solani* (Joy *et al*., 2004). Kumar and Tripathi, (1991) reported that leaf extracts of *Eupatorium cannabinum* exhibited complete toxicity against *Pythium* spp. The present study revealed that the chloroform extract of *P. minus* at 2 per cent concentration was found to be effective in checking the mycelial growth and spore germination of *A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici* under *in vitro* conditions.

CONCLUSION

All over the globe, people are searching for new botanicals to replace synthetics with a view to protect our environment. Irrational use of chemicals in agriculture adversely affects the health of living beings and soil too. Substituting the chemicals with botanicals by all possible means will pave way to reduce the residual effect of these chemicals in our environment and food products. Medicinal plants serve as the promising source of plant protection botanicals. Extensive research on medicinal plants to find out new lead molecules for plant protection measure will definitely save our mankind and environment.

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Table 1a. Antimicrobial activity of medicinal plants against plant pathogens

Sl.No	Plant Name	Diameter of inbibition zone in cm								
		A. helianthi			*A. solani*			*A. flavus*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
1.	*Abrus precatorius*	-	-	-	-	-	-	-	-	-
2.	*Adathoda bedonii*	-	-	0.4	-	0.3	-	-	-	-
3.	*Albizia lebbeck*	-	0.2	-	-	0.1	-	-	-	-
4.	*Alpinia calcarata*	-	-	-	-	-	-	-	-	-
5.	*Alstonia scholaris*	-	-	-	-	-	-	-	-	-
6.	*Alstonia venenata*	-	-	-	-	-	-	-	-	-
7.	*Anamirta cocculus*	-	0.5	-	-	0.4	-	-	0.3	-
8.	*Andrographis paniculata*	1.2	-	-	1.5	-	-	1.4	-	-
9.	*Anisomeles malabarica*	-	-	-	-	-	-	-	-	-
10.	*Aristolochia indica*	1.1	0.8	-	0.9	0.6	-	-	-	-
11.	*Artemisia parviflora*	-	0.6	-	-	0.8	-	-	-	0.5
12.	*Arundo donex*	-	-	-	-	-	-	-	-	-
13.	*Basella alba*	-	-	-	-	-	-	-	-	-
14.	*Begonia* sp.	-	-	-	-	-	-	-	-	-
15.	*Bixa orellana*	-	-	-	-	-	-	0.6	-	-
16.	*Blepharis maderaspatenis*	-	0.8	-	-	0.7	-	-	-	-
17.	*Caesalpinia sappan*	-	-	-	-	-	-	-	-	-
18.	*Calotropis gigantea*	1.2	-	-	1.0	-	-	0.8	-	-
19.	*Canthium dicoccum*	1.6	-	-	1.5	-	-	1.3	-	-
20.	*Capparis zeylanica*	-	-	-	-	-	-	-	-	0.8
21.	*Caralluma umbellata*	-	0.6	-	-	0.5	-	-	-	0.3
22.	*Cardiospermum halicacabum*	1.2	-	-	1.1	-	-	0.8	-	-
23.	*Cassia alata*	2.0	-	-	2.1	-	-	1.6	-	-
24.	*Cassia italica*	1.8	-	-	1.9	-	-	1.4	-	-
25.	*Cissampelos pareira*	-	-	-	-	-	-	-	-	-
26.	*Citrullus colocynthis*	0.5	-	-	0.4	-	-	-	-	-
27.	*Coccium decipiens*	-	-	-	-	-	-	-	-	-
28.	*Cocculus* sp.	-	0.3	-	-	0.4	-	-	0.4	-
29.	*Coix lacrymajobi*	-	-	-	-	-	-	-	-	-
30.	*Coscinium fenestratum*	-	-	0.7	-	-	0.8	-	-	0.5
31.	*Costus pictus*	-	0.4	-	-	0.5	-	-	-	-
32.	*Costus speciosus*	-	0.5	-	-	0.5	-	-	-	-
33.	*Crinum latifolium*	-	-	-	-	-	-	0.4	-	-

Sl.No	Plant Name	Diameter of inbibition zone in cm								
		A. helianthi			*A. solani*			*A. flavus*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
34.	*Crotalaria pallida*	-	-	-	-	-	-	-	1.0	-
35.	*Cryptolepis buchananii*	1.8	-	-	1.6	-	-	1.3	-	-
36.	*Datura metel*	1.1	0.8	-	1.2	1.0	-	-	-	-
37.	*Dioscorea* sp.	-	-	-	-	-	-	-	-	-
38.	*Emilia sonchifolia*	-	-	0.3	-	-	0.4	-	-	-
39.	*Garcinia gummigutta*	-	-	-	-	-	-	-	-	-
40.	*Glinus lotoides*	-	-	-	-	-	-	-	-	-
41.	*Gymnema sylvestris*	1.1	-	-	0.9	-	-	-	0.6	-
42.	*Hemidesmus indicus*	-	-	-	-	-	-	-	-	-
43.	*Hugonia mystax*	2.1	-	-	1.9	-	-	1.8	-	-
44.	*Hydnocarpus venenata*	1.3	-	-	1.0	-	-	0.4	-	-
45.	*Kedrostis* sp.	1.9	-	-	1.8	-	-	2.2	-	-
46.	*Leucas aspera*	-	-	0.6	-	-	0.5	-	-	0.4
47.	*Madhuca longifolia*	1.2	-	-	1.1	-	-	-	-	-
48.	*Mesua ferrea*	-	-	-	-	-	-	-	-	-
49.	*Morinda pubescens*	-	-	-	-	-	-	1.7	-	-
50.	*Mukia maderaspatana*	1.5	-	-	1.4	-	-	-	-	-
51.	*Oroxylum indicum*	0.3	-	-	0.2	-	-	-	-	-
52.	*Passiflora foetida*	-	-	-	-	-	-	-	-	-
53.	*Pergularia daemia*	0.8	-	-	0.9	-	-	-	0.4	-
54.	*Plumbago zeylanica*	2.1	-	-	2.0	-	-	1.8	-	-
55.	*Pogostemon heyneanus*	-	-	-	-	-	-	0.6	-	-
56.	*Polygonum minus*	2.2	2.6	-	2.7	3.3	-	2.5	3.1	-
57.	*Pterygota alata*	-	0.5	0.2	-	0.4	0.4	-	-	-
58.	*Rauvolfia tetraphylla*	1.2	-	-	1.3	-	-	0.8	-	-
59.	*Saccharum spontaneum*	-	0.3	-	-	0.2	-	-	0.3	-
60.	*Salvadora persica*	0.6	-	-	0.7	-	-	-	-	-
61.	*Sansevieria thysiflora*	-	-	0.8	-	-	0.5	-	-	-
62.	*Sapindus emarginatus*	-	-	0.6	-	-	0.7	-	-	0.7
63.	*Sapindus laurifolia*	-	-	0.5	-	-	0.6	-	-	0.5
64.	*Sarcostemma acidum*	1.3	-	-	1.2	-	-	1.0	-	-
65.	*Scilla hyacinthin*	-	0.6	-	-	0.5	-	-	0.2	-
66.	*Simarouba glauca*	-	-	-	-	-	-	-	-	-
67.	*Solanum trilobatum*	2.3	-	-	2.2	-	-	2.1	-	-
68.	*Talinum portulacifolium*	-	-	-	-	-	-	-	-	-
69.	*Terminalia arjuna*	-	0.8	-	-	0.7	-	-	0.3	-
70.	*Tinospora cordifolia*	2.1	-	-	1.9	-	-	2.2	-	-
71.	*Tithonia diversifolia*	-	-	-	-	-	-	-	-	-
72.	*Toddalia asiatica*	-	-	-	-	-	-	-	-	-
73.	*Tylophora indica*	0.3	-	-	0.5	-	-	-	-	-
74.	*Urginea indica*	-	-	-	-	-	-	-	-	-
75.	*Wrightia tinctoria*	-	-	-	-	-	-	-	-	-
76.	*Zehneria scabra*	0.6	-	-	0.7	-	-	0.4	-	-
77.	*Zizyphus oenoplia*	-	-	-	-	-	-	-	-	-

Sl.No	Plant Name	Diameter of inbibition zone in cm								
		A. helianthi			*A. solani*			*A. flavus*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
Ethanol		-			-			-		
Ketoconazole		4.0			3.8			3.5		

Ethanol – Negative control; Ketoconazole- Positive control
ME- Methanol extract; CE - Chloroform extract; PE – Petroleum ether extract

Table 1b. Antimicrobial activity of medicinal plants against plant pathogens

Sl. No	Plant Name	Diameter of inhibition zone in cm								
		A. niger			*C. musae*			*F. oxysporum* f.sp. *lycopersici*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
1.	*Abrus precatorius*	-	-	-	-	-	-	-	-	-
2.	*Adathoda bedonii*	0.5	-	-	0.2	-	-	0.3	-	-
3.	*Albizia lebbeck*	-	-	-	-	-	-	-	-	-
4.	*Alpinia calcarata*	-	-	-	-	0.2	-	-	-	-
5.	*Alstonia scholaris*	-	-	-	-	0.7	-	-	-	-
6.	*Alstonia venenata*	-	-	-	-	-	-	-	-	1.1
7.	*Anamirta cocculus*	-	0.3	-	-	-	-	-	-	-
8.	*Andrographis paniculata*	1.9	-	-	0.8	-	-	-	1.6	-
9.	*Anisomeles malabarica*	-	-	-	-	-	-	-	-	0.4
10.	*Aristolochia indica*	-	-	-	0.1	-	-	-	-	-
11.	*Artemisia parviflora*	-	-	0.4	-	-	0.5	-	-	-
12.	*Arundo donex*	-	-	-	-	-	-	-	0.6	-
13.	*Basella alba*	-	-	-	-	-	-	-	-	-
14.	*Begonia* sp.	-	-	-	-	-	-	-	-	-
15.	*Bixa orellana*	0.8	-	-	-	-	-	-	-	-
16.	*Blepharis maderaspatenis*	-	-	-	-	-	-	-	-	-
17.	*Caesalpinia sappan*	-	-	-	-	-	-	-	-	0.7
18.	*Calotropis gigantea*	0.7	-	-	-	0.9	-	-	0.5	-
19.	*Canthium dicoccum*	1.5	-	-	1.3	-	-	1.7	-	-
20.	*Capparis zeylanica*	0.8	-	0.7	-	-	-	-	-	-
21.	*Caralluma umbellata*	-	-	0.2	-	-	-	-	-	-
22.	*Cardiospermum halicacabum*	-	-	0.6	-	-	-	-	-	0.9
23.	*Cassia alata*	1.5	-	-	0.5	-	-	1.3	-	-
24.	*Cassia italica*	1.3	-	-	0.7	-	-	0.9	-	-
25.	*Cissampelos pareira*	-	-	-	-	-	-	-	-	-
26.	*Citrullus colocynthis*	-	-	-	-	-	-	-	-	-
27.	*Coccium decipiens*	-	-	0.6	-	-	0.4	-	-	0.2
28.	*Cocculus* sp.	-	0.3	-	-	0.3	-	-	-	-
29.	*Coix lacrymajobi*	-	-	-	-	-	-	-	-	-
30.	*Coscinium fenestratum*	-	-	0.3	-	-	0.5	-	-	0.6
31.	*Costus pictus*	-	-	-	-	-	-	-	-	-

Sl. No	Plant Name	Diameter of inhibition zone in cm								
		A. niger			*C. musae*			*F. oxysporum* f.sp. *lycopersici*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
32.	*Costus speciosus*	-	-	-	-	-	-	-	-	-
33.	*Crinum latifolium*	0.3	-	-	0.7	-	-	0.8	-	-
34.	*Crotalaria pallida*	1.1	-	-	-	-	-	-	-	-
35.	*Cryptolepis buchananii*	1.6	-	-	-	-	-	1.8	-	-
36.	*Datura metel*	-	-	-	-	0.9	-	-	0.8	-
37.	*Dioscorea* sp.	-	-	-	0.8	0.9	-	1.3	1.4	-
38.	*Emilia sonchifolia*	-	-	-	-	-	-	-	-	-
39.	*Garcinia gummigutta*	-	-	-	-	-	0.5	-	-	0.6
40.	*Glinus lotoides*	-	-	-	-	-	-	-	-	-
41.	*Gymnema sylvestris*	-	0.3	-	-	-	0.4	-	-	0.3
42.	*Hemidesmus indicus*	-	-	-	-	-	-	-	-	-
43.	*Hugonia mystax*	2.4	-	-	-	-	1.8	-	-	-
44.	*Hydnocarpus venenata*	-	-	-	-	-	-	-	-	-
45.	*Kedrostis* sp.	2.3	-	-	1.1	-	-	1.0	-	-
46.	*Leucas aspera*	-	-	0.2	-	-	0.5	-	-	0.4
47.	*Madhuca longifolia*	-	-	-	-	-	-	-	-	-
48.	*Mesua ferrea*	-	-	-	-	-	-	-	-	-
49.	*Morinda pubescens*	1.9	-	-	1.9	-	-	2.1	-	-
50.	*Mukia maderaspatana*	-	-	-	0.8	-	-	-	-	-
51.	*Oroxylum indicum*	-	-	-	-	-	0.7	-	-	0.6
52.	*Passiflora foetida*	-	-	-	-	0.3	-	-	-	-
53.	*Pergularia daemia*	-	0.3	-	-	-	0.1	0.4	-	-
54.	*Plumbago zeylanica*	1.6	-	-	-	0.4	-	2.1	-	-
55.	*Pogostemon heyneanus*	-	-	-	-	-	-	-	-	-
56.	*Polygonum minus*	3.0	3.2	-	1.1	1.0	-	3.1	3.4	-
57.	*Pterygota alata*	-	-	-	-	-	-	-	-	-
58.	*Rauvolfia tetraphylla*	-	-	-	-	0.4	-	-	0.5	-
59.	*Saccharum spontaneum*	-	0.2	-	-	-	-	-	-	-
60.	*Salvadora persica*	-	-	-	0.3	-	-	0.2	-	-
61.	*Sansevieria thysiflora*	-	-	-	-	-	-	-	-	-
62.	*Sapindus emarginatus*	-	-	0.5	1.2	-	-	1.3	-	-
63.	*Sapindus laurifolia*	-	-	0.4	1.0	-	-	0.9	-	-
64.	*Sarcostemma acidum*	0.8	-	-	0.7	-	-	0.8	-	-
65.	*Scilla hyacinthin*	-	0.1	-	-	-	-	-	-	-
66.	*Simarouba glauca*	-	-	-	-	-	-	-	-	-
67.	*Solanum trilobatum*	2.2	-	-	-	-	-	2.1	-	-
68.	*Talinum portulacifolium*	-	-	-	-	1.1	-	-	1.0	-
69.	*Terminalia arjuna*	-	0.2	-	-	-	0.4	-	-	0.9
70.	*Tinospora cordifolia*	2.1	-	-	1.1	-	-	2.0	-	-
71.	*Tithonia diversifolia*	-	-	-	-	-	-	-	-	-
72.	*Toddalia asiatica*	-	-	-	-	-	-	-	-	-
73.	*Tylophora indica*	-	-	-	-	0.6	-	-	0.8	-

Sl. No	Plant Name	Diameter of inhibition zone in cm								
		A. niger			*C. musae*			*F. oxysporum* f.sp. *lycopersici*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
74.	*Urginea indica*	-	-	-	-	-	-	-	-	-
75.	*Wrightia tinctoria*	-	-	-	-	-	-	-	-	-
76.	*Zehneria scabra*	0.2	-	-	0.7	-	-	0.8	-	-
77.	*Zizyphus oenoplia*	-	-	-	-	-	-	-	-	-
Ethanol		-			-			-		
Ketoconazole		3.6			2.9			3.5		

Ethanol – Negative control; Ketoconazole- Positive control
ME- Methanol extract; CE - Chloroform extract; PE – Petroleum ether extract

Table1c. Antimicrobial activity of medicinal plants against plant pathogens

Sl. No	Plant Name	Diameter of inhibition zone in cm								
		M. phaseolina			*R. solani*			*S. rolfsii*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
1.	*Abrus precatorius*	-	-	-	-	0.3	-	-	0.2	-
2.	*Adathoda bedonii*	0.2	-	-	-	-	-	-	-	-
3.	*Albizia lebbeck*	-	0.6	-	-	0.7	-	-	-	-
4.	*Alpinia calcarata*	-	-	1.0	-	-	1.1	-	-	-
5.	*Alstonia scholaris*	-	-	-	-	-	0.2	-	-	0.3
6.	*Alstonia venenata*	-	-	-	-	-	0.9	-	-	0.8
7.	*Anamirta cocculus*	-	-	-	-	-	-	-	-	-
8.	*Andrographis paniculata*	0.4	-	-	0.5	-	-	0.7	-	-
9.	*Anisomeles malabarica*	-	1.1	-	-	0.9	-	-	1.2	-
10.	*Aristolochia indica*	-	-	0.9	-	-	0.8	-	-	0.8
11.	*Artemisia parviflora*	1.1	-	-	1.0	-	-	0.7	-	-
12.	*Arundo donex*	-	0.6	-	-	0.5	-	-	0.5	-
13.	*Basella alba*	-	-	-	-	-	-	-	-	-
14.	*Begonia* sp.	-	-	-	-	-	-	-	-	-
15.	*Bixa orellana*	-	-	1.1	-	1.0	-	-	-	0.5
16.	*Blepharis maderaspatenis*	-	-	-	-	-	-	-	-	-
17.	*Caesalpinia sappan*	-	1.9	-	-	2.0	-	-	1.7	-
18.	*Calotropis gigantea*	1.8	-	-	1.7	-	-	2.0	-	-
19.	*Canthium dicoccum*	2.0	-	-	2.1	-	-	1.8	-	-
20.	*Capparis zeylanica*	-	-	-	-	-	-	-	-	-
21.	*Caralluma umbellata*	-	-	-	-	-	-	-	-	-
22.	*Cardiospermum halicacabum*	-	-	0.8	-	-	1.1	-	-	1.3
23.	*Cassia alata*	2.4	-	-	2.2	-	-	1.8	-	-
24.	*Cassia italica*	2.1	-	-	2.0	-	-	1.9	-	-
25.	*Cissampelos pareira*	-	-	-	-	-	-	-	-	-
26.	*Citrullus colocynthis*	-	-	-	-	-	-	-	-	-
27.	*Coccium decipiens*	1.0	-	-	1.1	-	-	0.9	-	-

Sl. No	Plant Name	Diameter of inhibition zone in cm								
		M. phaseolina			*R. solani*			*S. rolfsii*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
28.	*Cocculus* sp.	-	-	-	-	-	-	-	-	-
29.	*Coix lacrymajobi*	-	-	-	-	-	-	-	-	-
30.	*Coscinium fenestratum*	-	0.8	-	-	0.9	-	-	1.1	-
31.	*Costus pictus*	1.1	-	-	1.0	-	-	0.9	-	-
32.	*Costus speciosus*	1.2	-	-	1.3	-	-	1.0	-	-
33.	*Crinum latifolium*	-	-	-	-	-	-	-	-	-
34.	*Crotalaria pallida*	-	1.4	-	-	1.6	-	-	1.5	-
35.	*Cryptolepis buchananii*	1.7	-	-	2.1	-	-	1.9	-	-
36.	*Datura metel*	-	-	1.1	-	-	1.2	-	-	0.9
37.	*Dioscorea* sp.	1.2	1.1	-	0.8	-	-	1.3	1.2	-
38.	*Emilia sonchifolia*	-	-	-	-	-	-	-	-	-
39.	*Garcinia gummigutta*	-	-	0.9	-	-	0.9	-	-	1.1
40.	*Glinus lotoides*	-	-	-	-	-	-	-	-	-
41.	*Gymnema sylvestris*	-	0.8	-	-	0.9	-	-	0.6	-
42.	*Hemidesmus indicus*	-	-	-	-	-	-	-	-	-
43.	*Hugonia mystax*	1.7	-	-	2.2	-	-	2.1	-	-
44.	*Hydnocarpus venenata*	-	1.1	-	-	1.2	-	-	0.8	-
45.	*Kedrostis* sp.	2.5	-	-	2.1	-	-	1.6	-	-
46.	*Leucas aspera*	-	-	-	-	-	-	-	-	-
47.	*Madhuca longifolia*	-	-	-	-	-	-	-	-	-
48.	*Mesua ferrea*	1.1	-	-	1.2	-	-	1.0	-	-
49.	*Morinda pubescens*	-	-	0.8	-	-	0.7	-	-	0.2
50.	*Mukia maderaspatana*	-	-	-	-	-	-	-	-	-
51.	*Oroxylum indicum*	-	-	1.2	-	-	-	-	-	1.1
52.	*Passiflora foetida*	-	-	-	-	-	-	-	-	-
53.	*Pergularia daemia*	1.8	-	-	1.7	-	-	1.6	-	-
54.	*Plumbago zeylanica*	2.6	-	-	1.5	-	-	1.9	-	-
55.	*Pogostemon heyneanus*	-	-	0.6	-	-	0.5	-	-	0.4
56.	*Polygonum minus*	1.0	-	-	1.2	-	-	1.6	-	-
57.	*Pterygota alata*	-	-	-	-	-	-	-	-	-
58.	*Rauvolfia tetraphylla*	1.0	-	-	0.9	-	-	1.2	-	-
59.	*Saccharum spontaneum*	-	-	-	-	-	-	-	-	-
60.	*Salvadora persica*	-	0.8	-	-	0.9	-	-	1.5	-
61.	*Sansevieria thysiflora*	-	0.6	-	-	1.2	-	-	0.7	-
62.	*Sapindus emarginatus*	-	-	0.4	-	-	0.7	-	-	0.6
63.	*Sapindus laurifolia*	-	-	0.3	-	-	0.9	-	-	-
64.	*Sarcostemma acidum*	1.0	-	-	1.1	-	-	1.2	-	-
65.	*Scilla hyacinthin*	1.2	-	-	-	0.8	-	-	0.6	-
66.	*Simarouba glauca*	-	0.5	-	-	0.4	-	-	0.3	-
67.	*Solanum trilobatum*	1.2	-	-	1.3	-	-	1.0	-	-
68.	*Talinum portulacifolium*	-	-	-	-	-	-	-	-	-
69.	*Terminalia arjuna*	-	1.3	-	-	1.0	-	-	1.1	-
70.	*Tinospora cordifolia*	1.9	-	-	1.7	-	-	1.6	-	-

Sl. No	Plant Name	Diameter of inhibition zone in cm								
		M. phaseolina			*R. solani*			*S. rolfsii*		
		ME	CE	PE	ME	CE	PE	ME	CE	PE
71.	*Tithonia diversifolia*	-	-	-	-	-	-	-	-	-
72.	*Toddalia asiatica*	-	-	-	-	-	-	-	-	-
73.	*Tylophora indica*	-	-	-	-	-	-	-	-	-
74.	*Urginea indica*	0.5	-	-	0.4	-	-	0.6	-	-
75.	*Wrightia tinctoria*	-	-	-	-	-	-	-	-	-
76.	*Zehneria scabra*	-	-	0.2	-	-	0.1	-	-	0.3
77.	*Zizyphus oenoplia*	0.4	-	-	0.3	-	-	0.5	-	-
Ethanol		-			-			-		
Ketoconazole		2.9			3.1			3.0		

Ethanol – Negative control; Ketoconazole- Positive control

ME- Methanol extract; CE - Chloroform extract; PE – Petroleum ether extract

Table 2. Minimum inhibitory concentration for methanol, chloroform and petroleum ether leaf extracts of *P. minus* against plant pathogens

Contents	Dilution 1 (10mg/ml)			Dilution 2 (1mg/ml)			Dilution 3 (0.1mg/ml)			Dilution 4 (0.01mg/ml)		
	ME	CE	PE	ME	CE	PE	ME	CE	PE	ME	CE	PE
A. helianthi	NG	NG	G	G	G	G	G	G	G	G	G	G
A. solani	NG	NG	G	G	G	G	G	G	G	G	G	G
A .flavus	NG	NG	G	G	G	G	G	G	G	G	G	G
A. niger	NG	NG	G	G	G	G	G	G	G	G	G	G
C. musae	G	G	G	G	G	G	G	G	G	G	G	G
F.oxysporum fsp. *lycopersici*	NG	NG	G	G	G	G	G	G	G	G	G	G
M. phaseolina	G	G	G	G	G	G	G	G	G	G	G	G
R. solani	G	G	G	G	G	G	G	G	G	G	G	G
S. rolfsi	G	G	G	G	G	G	G	G	G	G	G	G
Solvent control	G			G			G			G		
Ketoconazole	NG			NG			G			G		
Cells	G			G			G			G		

ME – Methanol Extract; CE – Chloroform Extract; PE – Petroleum ether Extract

NG – No growth; G – Growth

Table 3. Effect of chloroform extract of *P. minus* on mycelial dry weight of fungal pathogens

Treatment	Mycelial dry weight							
	*A. solani**		*A. flavus**		*A. niger**		*F. oxysporum* f.sp. *lycopersici**	
	A	B	A	B	A	B	A	B
CE 0.50%	35.24	83.25	41.35	81.29	42.80	82.43	30.70	82.84

CE 1.00%	25.20	88.03	26.70	87.92	27.25	88.81	33.13	81.48
CE 1.50%	8.12	96.14	9.70	95.61	9.82	95.97	8.21	95.41
CE 2.00%	6.37	96.97	7.60	96.56	7.40	96.96	6.45	96.39
CE 2.50%	6.35	96.98	7.58	96.57	7.39	96.96	6.46	96.38
Control	210.50		221.00		243.60		178.90	

CE- Chloroform extract of *P. minus*

A- Mycelial dry weight (mg); B- Percent reduction over control

*Mean of four replications

Table 4. Effect of chloroform extract of *P. minus* on spore germination of fungal pathogens

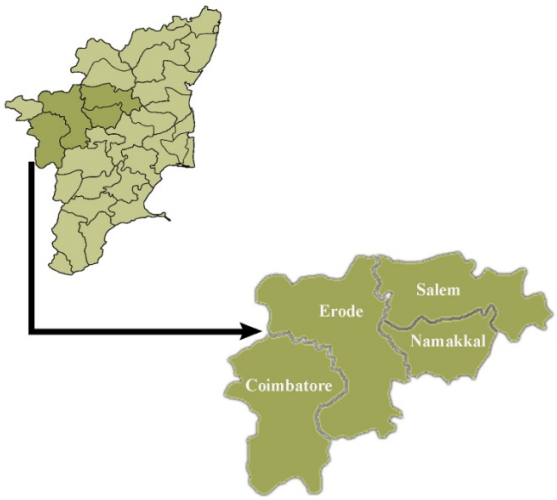
Treatment	Spore germination							
	A. solani*		***A. flavus****		***A. niger****		***F. oxysporum* f.sp. *lycopersici****	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
CE 0.50%	4.33	94.60	6.72	91.96	7.05	91.81	4.18	94.74
CE 1.00%	3.90	95.13	4.31	94.85	4.40	94.89	3.50	95.60
CE 1.50%	2.10	97.38	2.56	96.94	2.60	96.98	1.80	97.73
CE 2.00%	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
CE 2.50%	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Mancozeb (0.2%)	4.50	94.38	6.52	92.21	6.87	92.02	4.42	94.44
Control	80.12		83.65		86.10		79.50	

CE- Chloroform extract of *P. minus*

A- Spore germination (%); B- Percent reduction over control

*Mean of three replications

Plate 1. Places of survey in Tamil Nadu



Districts surveyed for collection of medicinal plants for study

Plate 2. Medicinal plants collected for study

Abrus precatorius *Adathoda bedoni* *Albizia lebbeck* *Alpinia calcarata* *Alstonia scholaris*

Alstonia venenata *Anamirta cocculus* *Andrographis paniculata* *Anisomeles malabarica* *Aristolochia indica*

Artemisia parviflora *Arundo donex* *Basella alba* *Begonia sp.* *Bixa orellana*

Blepharis maderaspatensis *Caesalpinia sappan* *Calotrophis gigantea* *Canthium dicoccum* *Capparis zeylanica*

Caralluma umbellata *Cardiospermum halicabium* *Cassia alata* *Cassia italica* *Cissampelos pariera*

Citrullus colocynthis *Coccium decipiens* *Cocculus* sp. *Coix lacrymajobi* *Coscinium fenestratum*

Plate 2 (Contd.,). Medicinal plants collected for study

Costus pictus | *Costus speciosus* | *Crinum latifolium* | *Crotalaria pallida* | *Cryptolepis buchananii*

Datura metel | *Dioscorea* sp. | *Emilia sonchifolia* | *Garcinia gummigata* | *Glinus latoides*

Gymnema sylvestris | *Hemidesmus indicus* | *Hugonia mystax* | *Hydnocarpus venenata* | *Kedrostis* sp.

Leucas aspera | *Madhuca longifolia* | *Mesua ferrea* | *Morinda pubescens* | *Mukia maderaspatana*

Oroxylum indicum | *Passiflora foetida* | *Pergularia daemia* | *Plumbago zeylanica* | *Pogostemon heyneanus*

Polygonum minus | *Pterygota alata* | *Rauvolfia tetraphylla* | *Saccharum spontaneum* | *Salvadora persica*

Plate 2 (Contd.,). Medicinal plants collected for study

Sansevieria thyrsifolia

Sapindus emarginatus

Sapindus laurifolia

Sarcostemma acidum

Scilla hyacinthin

Simarouba glauca

Solanum trilobatum

Talinum portulacifolium

Terminalia arjuna

Tinospora cordifolia

Tithonia diversifolia

Toddalia asiatica

Tylophora indica

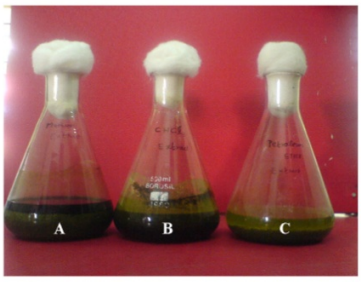
Urginea indica

Wrightia tinctoria

Zehneria scabra

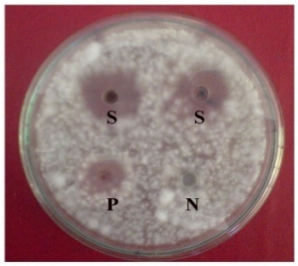
Zizyphus oenoplia

Plate 3. Preparation of plant extracts with solvents of various polarity

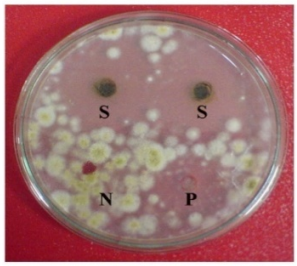


A - Methanol
B - Chloroform
C - Petroleum ether

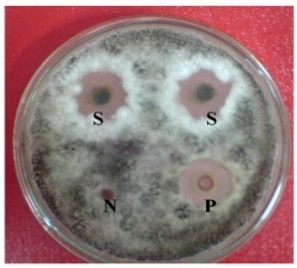
Plate 4. Antimicrobial activity of chloroform extract of *P. minus* against plant pathogens by agar well diffusion assay



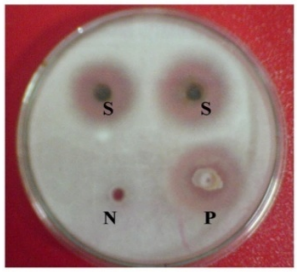
Alternaria solani



Aspergillus flavus



Aspergillus niger



Fusarium oxysporum* f.sp. *lycopersici

S - Sample (Chloroform extract of *P. minus*)
P - Positive control (Ketoconazole)
N - Negative control (100% Ethanol)

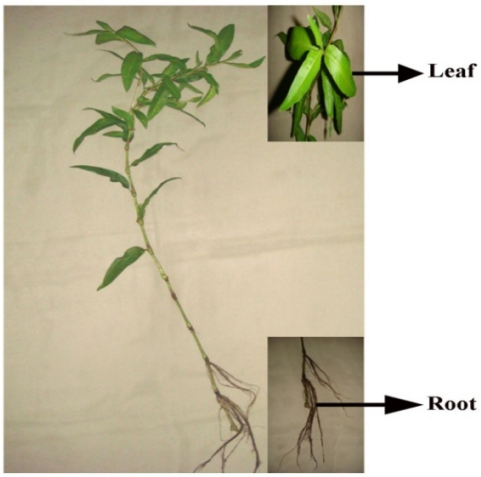


Plate 5. Effect of medicinal plants sample *Polygonum minus* Huds.

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No. BSI/SC/5/23/07-08/Tech. – 1502 Date: 05.02.2008

To
Ms. R. Parimala Devi,
Research Scholar,
Department of Agricultural Microbiology,
Tamil nadu Agricultural Unviersity,
Coimbatore – 641 003.

Madam,

The plant specimen brought for identification is determined as *Polygonum minus* Huds. - POLYGONACEAE

Yours sincerely,

05/02/08

(G.V.S. Murthy)
Joint Director

Plate 6. Certification of *Polygonum minus* Huds.

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RESEARCH ARTICLE

A Meticulous Investigation on Antidiabetic Profile of *Mimosa pudica*: the Shy Plant

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ABSTRACT

Mimosa pudica is a commonly neglected weed that grows in dumps, wastelands, roadside, walkway, marsh and hillside areas. The herb is popular in India in the names of thottal vadi, lajwanti etc., and is commonly available throughout the country. Mimosa has wider acceptance in the world of traditional medicinal system and has been used against various ailments like antidiabetic, wound healing, anti-venom, anti-arthritic, anti-convulsant, anti-malarial, anti-microbial, anti-fertility, etc from the time immemorial. So far some of the pharmacological actions are scientifically proven and some are still needed to be validated. Mimosa is reported with several phytochemicals like; alkaloids, terpenoids, glycosides, glycoproteins, flavonoids, carotenoids, coumarins, tannins, phenolic compounds, etc. Leaf and whole plant of Mimosa are highly praised for its significant antidiabetic potential. And the proposed mechanisms for the antidiabetic activity of Mimosa to control postprandial glucose are; stimulation of the remnant beta cells to secrete more insulin, regulation of the functions of metabolizing enzymes like glycogen synthase and the inhibition of carbohydrate metabolizing enzymes Besides that the binding, disintegrating abilities of Mimosa seeds are also scientifically validated. Therefore this herb can be proposed as an alternative to the present synthetic medicinal agents and to the existing excipients in drugs. Several marketed formulations of Mimosa are available nowadays which ranges from natural

herbal supplements to beauty care products. The ease of availability and being cheaper, further researches on this herb will make a revolution in the medicinal world and will reduce the burdens of patients in the future in all aspects.

Keywords: *Mimosa pudica,* Thottal Vadi, Traditional medicine, Antidiabetic activity, Herbal excipient.

INTRODUCTION

Diabetes mellitus is a cluster of metabolic disorders characterized by the disturbances in carbohydrate, protein, and lipid metabolism and is followed by microvascular and macrovascular complications. The global prevalence of diabetes mellitus is escalating presently. This disease is a threat to humanity because it affects the emotional, social well-being, and the quality of life of a person. The current treatment strategy including the oral hypoglycaemic agents, insulin, their associated adverse effects, lifestyle modifications, and the costly treatments demands herbal remedies which will be safe, less toxic, and cheaper than synthetic agents. *Mimosa pudica* (*M. pudica*) is one such herbal medicinal plant that is a part of common life. In spite of being a neglected weed, it can be a boon to diabetic patients in the future. It is an annual or perennial herb belonging to the family of Mimosaceae, commonly found in moist waste ground, lawns, and open plantation and weedy thickets, which grow throughout India. It is traditionally used for the treatment of many diseases.

The name Mimosa is derived from a Greek word; mimos (meaning mimic) pointing out to the fact that the leaves move in response to something moving against them and pudica is a Latin word, meaning bashful or shrinking to contact. The sensitive plant is usually distributed in the tropics. It is a well-known medicinal plant, and all the parts of this plant have reported medicinal property. Leaf and whole plants have been already reported for the antidiabetic activity while some are still not reported for this activity. Phytochemical screening has revealed the presence of several active constituents like alkaloids, glycosides, carbohydrate, steroids, flavonoids, phenols, resin, triterpene, and c-glycoflavines which can be a reason for the antidiabetic activity. Traditionally Mimosa is being used in diabetes, insomnia, hematuria, inflammation, emesis, dysmenorrhoea, menorrhagia, arthritis rheumatoid, convulsion, depression, etc. By combining the traditional knowledge with modern science and technology and by considering all the facts above, the development of an antidiabetic herbal formulation with superior efficacy and safety will set a milestone in the current treatment strategies of diabetes mellitus. Hence the objective of this review work is to evaluate the antidiabetic potential of *Mimosa pudica* by combining the traditional knowledge and in vivo antidiabetic studies with other pharmacological activities through various pieces of literature [1, 2, 3, 4, 5].

METHODOLOGY

The main sources of various research and review articles are from Google Scholar, pub med, ScienceDirect, researchgate, etc. Research articles having both in-vitro and in-vivo studies and the traditional uses of *Mimosa pudica* have been chosen for the work.

SYNONYMS

Mimosa andreana, Mimosa endymionis, Mimosa hirsute, Mimosa hispidula, Mimosa irritabilis [8] .

GENERAL CHEMICAL CONSTITUENTS PRESENT

Tannins, alkaloids, terpenoids, flavonoids, sterols, proteins, phenolic compounds, carbohydrate, steroids, glycoside, and saponins [9, 10] .

MORPHOLOGY

The leaves are small leaflets on the stalk and on touch, fold together. The stems are branched, with bristly hairs. It's predicted height is about 1 m when supported on other vegetation and more than 2 m in horizontal extension. The stems are reddish-brown and woody in nature and are sparsely or densely armed with curved prickles. The root system consists of a taproot and extensive fibrous roots with nodules. Appears with fine, flexible twigs and supported leaves with one or two pairs of pinnae and 15 to 25 pairs of oblong leaflets 3 to 12 mm long. The Mimosa possesses pink flowers that are clustered in globose heads. The legume (pod) is linear-oblong in nature, with 1.0 to 1.5 cm length and 3 mm width, and is presented with bristles on the margins. The pods appear in groups and contain two to four brown seeds [3, 12].

ECOLOGY

Mimosa pudica L. grows on most well-drained soils, even scalped or eroded subsoils and soils with low nutrient concentrations. It requires disturbed soils to establish itself. Repeated burning may encourage its spread in pastures. This undershrub is shade intolerant and does not compete with tall vegetation or grow under forest canopies. The 'pudica' species's roots produce carbon disulfide, which selectively inhibits colonization of the rhizosphere by various fungi like; mycorrhizal and pathogenic fungi. This plant occurs in croplands, orchards, pastures, mowed areas, roadsides, and areas disturbed by construction. Mimosa may grow as a single plant or maybe in tangled thickets. It grows from near sea level up to 1,300 m in elevation and especially in areas having annual precipitations from about 1000 to over 2000 mm. also the species is frost-sensitive [12].

ROLE OF *Mimosa pudica* IN VARIOUS SYSTEMS OF MEDICINE

siddha system of medicine

Thottal Vadi Choornam (powder form of *Mimosa pudica*) has been used widely in the Siddha system of medicine for the treatments of various ailments. Especially Leaves and roots are used in this system for treating diabetes mellitus, glandular swellings. Leaf juice of Mimosa is used as an eye drops to treat cataract. And decoction of Mimosa is applied externally for renal colic [4].

Contemporary medicine

In contemporary medicine, *Mimosa pudica* is being well investigated for its potential to yield several novel chemotherapeutic compounds. An alkaloid; mimosine present in Mimosa has been found to have potent antiproliferative and apoptotic effects. In addition to that, it appears to inhibit the myotoxicity and enzyme activity of cobra venom [4].

Traditional system of medicine

Mimosa has a major contribution to the traditional system of medicine around the world. All parts of this herb are being used or prescribed by various local or tribal healers against various ailments. And Kerala plays a significant role in using *Mimosa pudica* as a herbal ingredient in the formulations for the management of Diabetes mellitus.

LITERATURE REVIEW

Mimosa is a well-recognized herb in the traditional system of medicine for its innumerable pharmacological actions. The herb is attributed to various pharmacological actions like; Antidiabetic, Hypolipidemic, Antimicrobial, Antioxidant, Antihyperuricemic, Anti-convulsant, Anti-fertility, Digestive enzyme inhibitory effect, Antivenom activity, Anthelmintic, Antidepressant, Wound healing activity, etc.

Antidiabetic potential of *Mimosa pudica*

Antidiabetic activity of leaf, whole plant, and root parts of *Mimosa pudica* is already scientifically proven. All parts have produced a considerable antidiabetic activity when administered as ethanolic, petroleum ether extracts, and as dry powder. Mostly used part for the management of diabetes is the leaf. But the whole plant of Mimosa was reported with substantial antidiabetic activity when it was given in dry powder form than leaves. That is choornam of the whole plant of Mimosa could bring about a 61% reduction in the blood glucose level when compared to standard (66%). Proposed mechanisms for the antidiabetic activity of Mimosa are; either it stimulates the remnant beta cells to secrete more insulin, regulates the functions of metabolizing enzymes like glycogen synthase, or by the inhibition of carbohydrate metabolizing enzymes such as α-amylase and α-glucosidase, etc.

Hypolipidemic activity

The chloroform extract of *Mimosa pudica* leaves at a dose of 200 mg/kg body weight per oral has been screened for its hypolipidemic activity. The standard drug administered was Atorvastatin at a dose of 1.2 mg/kg body weight per oral. The measure of hypolipidemic activity got analyzed by inducing hyperlipidemia with the help of an atherogenic diet in Wistar albino rats and serum levels of various biochemical parameters such as total cholesterol, triglycerides, LDL, VLDL, and HDL cholesterol were determined. Administration of chloroform extract at the dose of 400 mg/kg showed a significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL, and an increase in HDL level which was similar to the standard drug; Atorvastatin, and was almost near the levels of normal control. The percentage of protection against hyperlipidemia in the plant extract-treated group was 63.3%, whereas the standard group protection is 68%, which further confirms the significant protective effect of the plant extract against hyperlipidemia [17].

Antihyperuricemia activity

Performed to screen anti-hyperuricemia activity of herbs extract of *Mimosa pudica* Leaf through analgesic and anti-inflammatory assays on mice. Hyperuricemia is indicated by pain and edema which are the symptoms of inflammation. Analgesic activity of *Mimosa pudica* Leaf herb extract at a dosage of 125, 250, and 500 mg/kg of body weight was observed on mice using the writhing reflex method with acetic acid 0.07 % as inducer. The results showed that all three dosages inhibited pain at a percentage of 9.58, 45.35, and 60.28% respectively. An anti-inflammatory activity assay was done using the carrageenan-induced paw edema method on white male rats. The dosages used were 250, 500, and 1000 mg/kg of body weight. The results showed that all three dosages inhibited edema at the percentages of 35.20, 42.74, and 51.10% respectively [5].

Anticonvulsant activity

Ethanolic extract of root parts of *Mimosa pudica* (EMPR) was prepared by a continuous soxhletion. EMPR was administrated at doses of 1000, 2000 mg/kg body wt along with valproate orally to albino mice, and anti-epileptic activity was assessed by maximal electroshock (MES) and pentylenetetrazole (PTZ) induced seizure models. Annulment of tonic hind limb extension phase and an increase in seizure latency period, when compared to the control group, was considered as the degree of protection in MES and PTZ induced convulsion models respectively. EMPR in the dose of 1000 and 2000 mg/kg body wt of mice showed a significant anti-epileptic property in both MES and PTZ induced seizure models. There was a significant abolition of the tonic hind limb extension phase in the MES model. There was also a significant increase in the seizure latency period in PTZ induced seizure model [9].

Antioxidant properties

In vitro antioxidant activity of the hexane extract of Mimosa leaves was exerted by evaluating 2, 2-Diphenyl-Picrylhydrazyl (DPPH), hydroxyl, nitric oxide, and superoxide radicals. In addition, the total phenolic content also measured. The hexane extracts from *Mimosa pudica* Linn. revealed a significant scavenging effect on DPPH (IC_{50} 20.83 mM), hydroxyl (IC_{50} 19.37 mM), nitric oxide (IC_{50} 21.62 mM) and superoxide (IC_{50} 22.19 mM) radicals. In nitric oxide radical inhibition assay at a concentration of 21.62 mM of M. pudica hexane extract 50% of nitric oxide generated by

incubation was scavenged. The IC_{50} value of M. pudica hexane extract on superoxide scavenging activity was found to be 22.1 mM, whereas the IC_{50} values for Butylated Hydroxy Toluene (BHT) and vitamin C were found to be 21.7 and 22.6 mM, respectively [18].

Digestive enzymes inhibitory effect

α-amylase and α-glucosidase enzyme inhibitory assay were used to evaluate the antidiabetic potential of the *Mimosa pudica* extracts; as these enzymes are responsible for the post-prandial rise of blood glucose level in a person by breaking down polymeric macromolecules into their smaller building blocks, in order to facilitate their absorption by the body. The ethanolic extract of fresh aerial parts of *Mimosa pudica* prepared by maceration showed a higher in-vitro α-amylase (30.08±5.22) and α-glucosidase (38.29±2.01) % of inhibition than the standard; acarbose (28.24±13.66 and 36.93±2.70, respectively) and Supercritical fluid extract (15.67±4.03 and 28.36±2.01, respectively) at the concentration of 1 mg/ml. The ethanolic extract also showed better results for the presence of total phenolic and flavonoid contents in comparison to supercritical extract. These compounds could be responsible for the antidiabetic effect of *Mimosa pudica* by controlling post-prandial high blood sugar through digestive enzymes inhibitory mode of action [19].

Antifertility effect

Mimosa pudica is one of the folk medicinal plants commonly used as an antifertility agent in some places in India. The dried methanol extract of the root was administered orally to Swiss albino mice for 21 consecutive days at a dose of 300 mg/kg body weight/day. Estrous cycle, reproductive hormones (LH, FSH, prolactin, estradiol, and progesterone) and the number of litters produced were studied in both control and extract-administered groups. *M. pudica* root extract prolonged the length of the estrous cycle with a significant increase in the duration of the diestrous phase and reduced the number of litters in albino mice. The number of litters was increased in the post-treatment period. The analysis of the principal hormones (LH, FSH, prolactin, estradiol, and progesterone) showed that the root extract altered gonadotropin release and estradiol secretion. The decrease in FSH level in the proestrus and estrus stages in the extract-administered group compared with those of control animals indicates the disturbance of the estrous cycle and ovulation through suppression of FSH [20].

Anthelmintic activity

The study was undertaken to evaluate the anthelmintic activity of different extracts of seeds of *Mimosa pudica*. The different successive extracts used against Pheretima posthuma as a test worm were petroleum ether, ethanol, and water at different concentrations such as 100, 200, and 500mg/kg to determine paralysis and time of death of the worms. Albendazole was included as a standard reference (100, 200, 500mg/kg) and normal saline as control. Observations were based on the time taken to paralyze and death (when earthworms lost their motility followed by fading of body color). The result of the present study indicated that the crude alcoholic extract and aqueous extracts significantly demonstrated paralysis and also caused the death of the worms in a dose-dependent manner as compared to standard reference albendazole. While petroleum ether extracts shown weak anthelmintic effect compared to standard, ethanol, and aqueous extracts [21].

Antimicrobial

The antimicrobial activity of Mimosa was screened by testing against various microorganisms such as *Aspergillus fumigatus, Citrobacter divergens, and Klebsiella pneumonia.* The different concentrations used for the assessment of antimicrobial activity were 50, 100, and 200µg/disc. The results of the antimicrobial assay of the methanolic extract of *Mimosa pudica* leaves indicated that the plant exhibited antimicrobial activity against the tested microorganisms at three different concentrations of 50, 100, and 200µg/disc. A significant activity of the extract was observed against all the three microorganisms tested. The maximum zone of inhibition for the antimicrobial activity was obtained for *Aspergillus fumigatus* and *Klebsiella pneumonia* which was at a concentration of 200µg/200µl. At the same time,

Klebsiella pneumonia could produce a good antimicrobial activity against both the concentrations and *Citrobacter divergens* were able to produce resistance against *Mimosa pudica* extract at all concentrations [22].

Antidepressant actions

In this study, the behavioral actions of aqueous extracts of Mimosa at various concentrations were tested. Rats having received saline (0.9%; 0.30 ml; LP.), clomipramine, desipramine, or several dosages of aqueous extracts from Mimosa during a 30-day period were submitted to the forced swimming test and to the test for differential reinforcement of low rates of response at 72 seconds. Aqueous extracts were administered at a doses of; ml = 2.0 mg/kg; m2 = 4.0 mg/kg; m3 = 6.0 mg/kg; m4 = 8.0 mg/kg. Any possible anxiolytic action resulting from several doses (ml = 2.0 mg/kg; m2 = 4.0 mg/kg; m3 = 6.0 mg/kg; m4 = 8.0 mg/kg) of extracts of Mimosa were compared with those caused by diazepam (1.3 mg/kg, LR) in the elevated plus-maze test. Results showed that clomipramine (1.25 mg/kg, I.R), desipramine (2.14 mg/kg, I.R) and M. pudica (6.0 mg/kg and 8.0 mg/kg, I.R) reduced immobility in the forced swimming test and increased the rate of reinforcers received in the DRL-72s test; these data suggest that Mimosa produces antidepressant effects in the rat. In the elevated plus-maze test, diazepam showed an increase in the open-arms exploration time, but Mimosa did not show any comparable action at any of the tested doses. Therefore study can be concluded as Mimosa produced an antidepressant-like profile similar to the two tricyclic antidepressants mentioned above [23].

Against cobra venom

Studied the effectiveness of *Mimosa pudica* tannins (MPT) in neutralizing the lethality of Naja kaouthia (common cobra) venom, which was then compared with commercially derived tannins. Venom doses ranging from 0.7 to 1.1 mg/kg were injected into each group of mice. The two LD50 of Naja kaouthia venom was calculated to be 0.92 ± 0.10 mg/kg. Preincubation of 2 LD50 (1.84 mg/kg) of Naja kaouthia venom with Mimosa tannins was able to completely neutralize the snake venom since the survival rate of mice was 100% after 24 hours of observation. Preincubation of the venom with commercial tannic acid showed only a mouse survival rate of 12.5%. Tannins obtained from Mimosa were eight times more effective in neutralizing the lethal effects of Naja kaouthia venom compared to the tannic acid. The mouse group which did not undergo preincubation, no protection against the effects of the venom was observed. MPT was found to be more effective in neutralizing the lethality of Naja kaouthia venom when compared to commercial tannic acid. Two protein spots were not observed in the two-dimensional gel electrophoresis (2-DE) of the MPT treated mouse point outs the down-regulation of venom proteins. The results from this study indicated that tannins obtained from Mimosa are better than tannic acid in neutralizing the lethality of Naja kaouthia venom in vitro. But in the in-vivo experiment, only the serum with antivenom was able to fully protect the mice against Naja kaouthia venom-induced lethality after 24 hours. Administration of Naja kaouthia venom and MPT by separate routes failed to inhibit the lethality in mice. The same result was observed in mice treated with commercially derived tannic acid [24].

Wound healing activity

The study has been undertaken to examine the wound healing activity of the Ethanolic extract of leaves of *M. pudica* in excision and burn wound models. Healthy Sprague Dawley rats weighing 150-180 g were randomly distributed into four groups of 6 each in excision and of 6 each in burn wound models. Mupirocin used as the standard in both models. Drug-treated groups were applied with 5 % w/w & 10%w/w ointment in soft paraffin base leaves extract of *M. pudica* respectively and positive control group rats were applied with mupirocin ointment two times a day. The extract treated animals exhibited 73% & 92% respective reduction in the wound area when compared to control which was 28%. The wound contraction studies revealed that; as the concentration of herbal extract increases the wound contraction also. These preliminary results point out that, *M. pudica* facilitates wound healing by increasing the rate and extent of wound closure [25].

RESULT AND DISCUSSION

Based on the above results, a graph can be plotted by taking doses of plant parts in the form of extract or dry powder on the X-axis and their corresponding % blood glucose reduction on the Y-axis. Either Metformin or Glimepiride was the mostly administered standard drugs for the comparison with test drug; Mimosa. In most of the studies, alloxan was used to induce diabetes in the experimental animals at a dose of 150 mg/kg body weight. And most used animals in in-vivo studies were albino Wistar rats. Significant blood glucose reduction was observed when Mimosa gave as a dry powder form than the extracts. A dose-dependent increase in blood glucose reduction was evident when Mimosa root administered in dry powder form. But the choornam of the whole plant of Mimosa produced a notable result than dry powder of roots. A dose-dependent increase in the blood glucose reduction was evident in the case of choornam of the whole plant too. And the results were comparable to that of standard drugs. Standard drugs; glimepiride and metformin at lower doses couldn't produce a better result than Mimosa. Percentage blood glucose reduction for metformin (500mg/kg) was comparable but was less than that of dry powder of the whole plant of mimosa. Glibenclamide at a dose of 10mg/kg had the best result in blood glucose reduction than other samples.

The treatment with modern medicines like insulin therapy, sulfonylureas, thiazolidinediones, biguanides, etc., mostly accompanied by numerous side effects. Though, searching for new antidiabetic drugs from natural plants is still at a high rate because they are easily available in the communities and can alleviate side effects on diabetes mellitus. *Mimosa pudica* is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine for various diseases. They produce their antidiabetic action by different mechanisms like stimulating or regenerating the effect on β cell or extrapancreatic effect for hypoglycemic activity. Likewise, herbal formulation treatment can significantly alter the pattern of glucose tolerance in normal and diabetic rats. It is possible that herbal formulation may act through both, pancreatic and extrapancreatic mechanism(s) [26].

From the time immemorial, various herbs and the traditional medicinal system do have a crucial role in various aspects of life. *M. pudica* or touch-me-not herb is one such a herbal plant that is using traditionally for several ailments and is commonly available in India. Despite being a neglected weed, *M. pudica* possesses the significant antidiabetic potential to be proposed as a strong alternative medicinal source against the global threat of various synthetic antidiabetic agents. Being cheaper, easily available, and rich in numerous promising compounds, further studies and experimentations on this plant will aid to get newer antidiabetic agents from *M. pudica.* Along with the antidiabetic effect, the herb is traditionally acclaimed for several pharmacological actions. Some of it is scientifically proven and some are yet to be validated. Henceforth further meticulous researches works on this herb will pave a milestone as a medicinal alternative to the concurrent medicinal flora and to the treatment strategies to be a boon to mankind in the future [27].

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39. Herbo Natural Fertilex For Men(60 Capsules & 300g Powder) For ... https://www.saloni.pk/herbo-natural-fertilex-for-men-60-capsules-300g-powder/
40. Welex Ayucid for Hyperacidity and GERD - 60 vegetarian capsules by Ayucid. https://www.amazon.in/Welex-Ayucid-Hyperacidity-GERD-vegetarian/dp/B07BQ9L6LM
41. Ayuwelex Welex Ayurhoids Piles Care Capsules Ayurvedic Supplement -Pack of 60 Capsule by Ayuwelex. https://www.amazon.in/Ayuwelex-Ayurhoids-Capsules-Ayurvedic-Supplement/dp/B07HXZYHN6
42. Love Beauty and Planet Coconut Water & Mimosa Flower Shampoo ... https://www.lovebeautyandplanet.com/uk/hair/allhairproducts/shampoo/coconut-water-&-mimosa-flower-shampoo.html

TABLE NO: 1 COMMON NAMES [6, 7]

English	Touch Me Not Plant, Sleeping Grass, Sensitive Plant, Humble Plant, Shy Plant
Hindi	Lajwanti
Malayalam	Thottavadi,Thottalvadi,Theendarmani
Tamil	Thottalsenungi
Sanskrit	Ajalikalika
Kannada	Hadergitte
Telugu	Attapatti

TABLE NO: 2 TAXONOMY [11]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Subfamily	Mimosoideae
Genus	Mimosa
Species	*M. pudica*

TABLE NO: 3 ANTIDIABETIC FORMULATIONS PRESCRIBED BY LOCAL AND TRADITIONAL HERBAL HEALERS IN KERALA

ADDED HERBALS IN THE FORMULATION	MODE OF ADMINISTRATION
Kerala	
M. pudica (Thottavadi)	Plant juice is taken orally twice daily. [13]
Urumbikkara Hills, Idukki	
M. pudica (Thottavadi)	The whole plant extract is used as a remedy for Diabetes. [14]
Wayanad	
1) M. pudica (Thottavadi) - Roots + *Arenga wightii* (Jnetti) - Pith + *Areca catechu* (Adakka) - Roots	(i) - Crush 20g fresh young roots of Areca using wooden mortar & pestle to yield 10ml of juice on squeezing (ii) - Boil 15g dried crushed roots of *M. pudica* in 100ml of water for 2minutes (iii) - Add 10g of pith powder to Areca, *M. pudica* extract mixture (iv) - 3ml of above preparation is advised once in a day & taken for 1 month. [15]
2) *M. pudica* (Thottal vadi) – Roots + *Symplocos laurina* (Pachotti) – Bark + *Syzgium caryophyllum* (Njaral) – Bark	(i) - Take 10g of dried crushed bark of Symplocos, Syzgium & dried powdered roots of *M. pudica* in the fold of clean cotton cloth (ii) - Boil in 100ml water for 3minutes (iii) - 3ml of decoction is advised thrice in a day & continued for 2 weeks. [15]
3) *M. pudica* (Thottal vadi) - Roots + *Terminalia arjuna* (Neer maruth) - Bark + *Oryza sativa* (Nellu) - Rice bran	(i) - Take 10g crushed roots of *M. pudica* & dried crushed bark of Terminalia in the fold of clean cotton cloth & boil in water for 3minutes (ii) - Add about 300g of fine rice bran powder to the infusion & blend thoroughly (iii) - Roll the preparation on cleaned white palm to get a spherical tablet of 3g weight (iv) - Single tablet is advised to take twice in a day after food for 2 weeks. [15]
4) *M. pudica* (Thottal vadi) - Roots + *Terminalia chebula* (Kadukka) - Fruit pulp + *Butea monosperma* (Chami) - Flowers	(i) - Crush 20g of fully bloomed flowers of Butea in 20ml of water to get 10ml extract (ii) - Take 10g each of the dried crushed fruit wall of Terminalia & dried crushed roots of *M. pudica* in the fold of a clean cotton cloth (iii) - Boil in 200ml of water for 3 minutes to get the extract (iv) - Mix preparations i & ii (v) - 3ml of prepared mixture is advised to take thrice a day after meal & continued. [15]
5) M. pudica (Thottal vadi) - Whole plant + *Asparagus racemosus* (Sathavari) - Tuberous roots + *Calophyllum apetalum* (Punna) - Seeds	(i) - Crush 20g of each of the chopped whole plant of *M. pudica* & chopped dried tuber of Asparagus in 50ml of water to squeeze out 20ml of extract (ii) - Crush 10g dried crushed seeds of Calophyllum in 10ml of ethyl alcohol to separate the extract (iii) - Mix extracts i & ii (iv) - 3ml of mixture is advised to take thrice in a day after meal & continued. [15]

TABLE NO: 4 SUMMARY OF IN-VIVO ANTIDIABETIC STUDIES OF *Mimosa pudica*

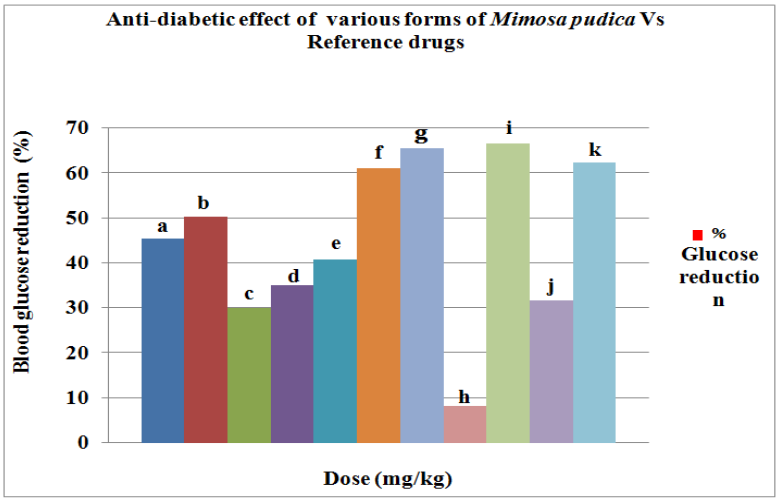
PLANT PART	ANIMAL/ INDUCTION	EXTRACT/ STANDARD DRUG	DOSE	% REDUCTION IN GLUCOSE	REFERENCE
Leaves	Alloxan induced (150mg/kg) Wistar rat (200-250g) of either sex	Petroleum ether Ethanolic extract Metformin	600 mg/kg/day 600 mg/kg 500 mg/kg b.wt.***	292.67±2.91 to 198.96±3.26 50.35% 62.44%	N.G.Sutar et al., 2009.[16]
Leaves	High Fat Diet and Low Dose **STZ (35mg/kg b.wt.***)-Induced male Albino Wistar Rats	Ethanolic extract Metformin	*DC 300 mg/kg Control rats;300 mg/kg 200 mg/kg	220±20 45.45% 61.36% 31.81%	Gunasekaran K et al., 2017.[3]
Whole plant	Alloxan induced Wistar rat (180-220g)	Thottal vadi Choornam Glibenclamide	*DC 100 mg/kg 200 mg/kg 10 mg/kg	280.67±4.66 61.16% 65.55% 66.62%	Viswanathan R et al., 2013.[4]

*DC- Diabetic Control **STZ – Streptozotocin ***b.wt. – Body Weight

TABLE NO: 5 AVAILABLE MARKETED FORMULATIONS OF *Mimosa pudica*

PRODUCT NAME	CATEGORY	MANUFACTURER
Thottasinungi powder 300g	Neotea	Neoteric India [28]
M. pudica seed 120 capsules	Fat-Soluble Dietary Supplement	Microbe Formulas [29]
M. pudica powder 112gms	Dietary Supplement	BioPure [30]
M. pudica capsules 180 X 1000mg	Herbal Supplement	Nutri Herbs [31]
LUCO PRAKSAH 30 capsules 500mg	Herbal supplement	Vitagreen [32]
Stonhills Kidney Support 60 tablets 400mg	Kidney Care Supplement	Herbal Hills [33]
Diabecon*30 tablets	Antidiabetic	Himalaya Herbal [34]
PARA 1 *M. pudica* Seeds 120 capsules	Dietary Supplement	Cellcore Biosciences [35]
M. pudica Seed Alcoholic Tincture 1:2 Extraction 125ml	Natural Herbal Supplement	Nutri Herb [36]
M. pudica supreme 90*400mg Vegetable capsules	Dietary Supplement	Supreme Nutrition [37]
M. pudica 1:2 oil infusion 125ml	Natural Herbal Supplement	Nutri Herb [38]
Fertilex 60 capsules & 300g powder	Men's Multivitamins	Herbo Natural [39]
AYUCID 60 capsules	Relieves hyperacidity & GERD	Welex Laboratories Pvt. Ltd. [40]

AYURHOIDS 60 capsules Love Beauty & Planet 400ml (with coconut water & *M. pudica* flower	Provide prompt relief from hemorrhoids Hair conditioner, Shampoo, Body lotion, Body wash	Welex Laboratories Pvt. Ltd. [41] Love Beauty & Planet [42]



a - leaf ethanolic extract 300mg/kg, b - leaf ethanolic extract 600mg/kg, c - root powder 2mg/kg, d - root powder 4mg/kg, e - root powder 6mg/kg, f - whole plant choornam 100mg/kg, g - whole plant choornam 200mg/kg, h - glimepiride 800 µg/kg, i - glibenclamide 10 mg/kg, j - metformin 200 mg/kg, k - metformin 500 mg/kg.

Figure no: 1 – percentage glucose reduction of *Mimosa pudica*

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RESEARCH ARTICLE

Bio Additive Development from *Madhuca indica* Oil, Engine Performance Evaluation and Emission Studies

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ABSTRACT

Energy consumption round the globe is turning away from mineral fuel because of volatile market price, depletion in oil reserve and environmental pollutions. Rapid urbanisation, impeccable growth of population and comfortable living style is ever augmenting the energy demand. At this exigency development of sustainable bio-additives for mineral diesel promises to meet a prorated energy demand around the world. Non-edible oils are of significant potential resources for developing bio-additives without endangering food chain security in developing countries like India. Successive acid and base catalyzed transesterification of non edible oils to produce bio-additives for mineral diesel has been industrially adopted for high yielding and less reaction time. Present work involves degumming of *Madhuca indica* oil followed by two steps esterification using methanol. The esterification process was mediated by H2SO4 and Na OH catalyst to produce bio-additive. The produced bio-additive was characterized in terms of physic-chemical properties following ASTM standards with evaluation of engine performance and emissions studies.

Keywords: *Madhuca indica,* environmental, Energy, pollution, emissions

INTRODUCTION

Madhuca indica is a perennial tree of Indian origin belonging to Sapotaceae family [1, 2, 3, 4]. It is grows in arid environments with evergreen foliage and attains a height of 20 m approximately [5, 6]. The annual production of seeds from *Madhuca indica* is of about 60 million tons in India. The tree starts production after 10 years and continues up to 60 years. The kernel volume is about 70% of the seed and bears 50% oil [7, 8, 9]. Annual production of individual tree is about 20–40 kg seeds depending on maturity and size. The total oil yield per hector is 2.7 tonne per

year. Approximate oil bearing capacity of seed is about 35–40% [10]. The *Madhuca indica* tree with different stages of maturity is shown in Fig-1. Considering the high oil bearing potential of *Madhuca indica* seeds the present work was motivated to develop bio-additive from this forest based resource. The investigation was carried out to develop bio-additive from *Madhuca indica* oil by degumming followed by two step esterification process. The modified oil was fuelled to a stationary single cylinder, four stroke diesel engines in various additive blends with mineral diesel to investigate the performance and emission characteristics considering mineral diesel as base line fuel. The engine trial data were interpreted graphically to compare with mineral diesel performance.

The additive blend of 20% with mineral diesel suffered no problem but the higher blends were associated with operational and durability problems when subjected to long-term engine trial. These problems were attributed to high viscosity, low volatility and poly unsaturated character of *Madhuca indica* oil. The present investigation also includes the possibility of replacing mineral diesel by fuelling modified additive in absolute mode with pros and cons. Most of the properties of *Madhuca indica* in bio-additive were comparable to mineral diesel. However the improvement of its cold condition flow characteristics was the major challenge to fuel it in absolute mode. *Madhuca indica* oil contains significant amount of saturated fatty acids that displays higher values of cold flow properties. Crystallization of the saturated fatty acid methyl esters in cold weather condition causes fuel glutinous and operability problems as solidified material clog fuel lines and filters. It has been well established that the presence of higher amount of saturated compounds were responsible for higher values of cold flow properties [11]. The engine trial for 200 hours by fuelling absolute *Madhuca indica* bio-additive resulted considerable clogged filter. However the same trial with fuelling mineral diesel showed considerably clean filter as displayed in Fig-2. The clogging of fuel filter may be minimised by pre heating of fuel tank,fuel line and using fuel filter heaters. Blending of cold flow improvers like anti-gel additives that enhance the impact of crystal morphology or blending with kerosene which causes freezing point depression mitigates the problem[12].

MATERIALS AND METHODS

Removal of gums and alkaloids

The crude *Madhuca indica* oil was centrifuged at 10000 rpm a REMI Model-24 centrifuge machine and the super natant oil was collected free from heavy contaminates.The crude oil (100 ml) was mixed with 25 ml methanolic H_3PO_4 solution (12%, v/v) to form a homogenious mixture and allowed to stand overnight. The oil was separated from methanol layer and precipitated compounds are filtered through silica gel (60–120 mesh) under suction. The filtrate consisted of methanol and phosphoric acid could be recycled three times for degumming Mahua oil. The reaction process was economically more viable.The degummed oil was mixed with 0.1% aqueous sodium hydroxide solution and kept overnight.Other day aqueous portion was discarded and oil was washed twice with hot water to remove residual alkali. Then oil was heated on boiling water bath for 1 hour and then passed through warmed (105^0 C in an oven before use) anhydrous Na_2SO_3 to remove moisture from oil. Resultant oil was stored as refined alkaloid-free degummed oil(DO) for further treatment.The degumming reaction process yielded 94% DO from crude mahua oil (CMO).

Two step esterification

The degummed oil (DO) was mixed with sulphuric acid and methanol in the ratio of 50:10:1 (oil: CH_3OH:H2SO4, v/v/v) and stirred mechanically at 1000 rpm at 65^0C for 3 hours. After completion of esterification process, two layers were separated within 30 min. The lower layer was discarded and followed by neutralization with methanolic costic soda solution and methanol was recovered from oil. The neutral oil was transesterified by mixing with sodium hydroxide and methanol in a ratio of oil:alkali:methanol (25:0.2:5) and stirred well mechanically at 800 rpm for 4 h at 55^0C . The desired transesterified oil was separated from lower layer by separating funnel and washed with hot

water twice to remove impurities and resultant transesterified oil (TEO) was stored for further analysis. After two-step transesterification 87% of the DO was converted to TEO.

ASTM D-97 pour point procedure [13]

The observation of the sample was started at a temperature 9^0C above the expected pour point. The sample was immersed into an -18^0C cooling bath.Readings were taken for every 3^0C decrease in the temperature until the sample totally ceased to flow (the sample was held in horizontal position for 5 s). Reading of the test thermometer was taken and added with 3^0 C as infered pour point (ASTM D-97).

ASTM D-2500 cloud point procedure [14]

The cloud point is the temperature at which a cloud of wax crystal first appears in a liquid when it is cooled under controlled conditions during a standard test. The same cooling procedure articulated in ASTM D-97 was followed; the samples were examined at intervals of 1^0C, until any cloud was observed at the bottom of the test jar. The cloud point was infered to the nearest 1^0C (ASTM D-2500).The experimental setup for cloud point was displayed in Fig.3.

Kinematic viscosity

In cold climatic condition the viscosity of *Madhuca indica* bio-additive was imperative, considering the spray characteristics of the injector, since the change in spray can greatly alter the combustion characteristics of the mixture. The esterification of *Madhuca indica* oil remarkably lowered the viscosity. It was revealed that the measured viscosity of *Madhuca indica* oil ester was little higher than mineral diesel over the higher range of temperatures considered. However the viscosity of bio-addditive in low temperature region was different in comparison to mineral diesel. High viscosity suffered problems in pumping and spray characteristics (atomization and penetration). The inefficient mixing of bioadditive with air contributed to incomplete combustion. However additive blends with mineral diesel like B-10, B-20 improved the viscosity.The variation of kinematic viscosity of mahua methyl ester(MME) and its additive blends with mineral diesel(B-10,B-20) was displayed in Fig.4.

Flash point and fire point

The flash and fire point was meaasured with a Cleave land apparatus. *Madhuca indica* bio-additive had higher flash and fire point. Hoever additive blends with mineral diesel or preheating improved the flash and fire point to a considerable amount.The comparative study in physico chemical properties of *Madhuca indica* bio-additive with mineral diesel and degummed oil was presented table-1.

Engine setup

A four stroke,water cooled,single cylinder engine coupled with edicurrent dynamometer was used for engine trial as displayed in Fig.5. The engine was computerised with engine soft equation solver to calculate the engine performance parameters. AVL make gas analyser was employed to detect the noxious emissions like, carbon dioxide,unburnt hydrocarbons,carbon monoxide and nitrous oxides including excess free oxygen supplied to engine.The engine trial was conducted by fuelling absolute *Madhuca indica* bio-additive and its additive blends B-10, B-20, B-30, B-50 and mineral diesel. The reference study was based on mineral diesel to interprete the data for comparision.The trial was conducted at 1500 rpm with varing loads.The engine specification was presented in table.2.

RESULTS AND DISCUSSION

Engine performances

Brake power

The variation of brake power with load by fuelling absolute *Madhuca indica* bio-additive, B-10, B-20, B-30, B-50 and mineral diesel was considered for present analysis as displayed in Fig-6. The brake power of petroleum diesel was found highest from part load to full load, and that of B-100 was found slightly lower addressing to high viscosity, low calorific value and poor spray characterics. The power loss in B-100 was 5% at full load and 18:1compression ratio; however B-10 displayed a loss of 1 to 2%. Hence 10% additive blend with mineral diesel can be recommended for fuelling diesel engines without any engine modification and adulteration.

Brake mean effective pressure

The variation of brake mean effective pressure with load by fuelling absolute *Madhuca indica* bio-additive, B-10, B-20, B-30, B-50, and mineral diesel was taken for analysis as presented in Fig-7. The BMEP of mineral diesel was found highest from part load to full load and that of B-100 was found lowest which was rationalised to higher viscosity, low calorific value and inferior atomisation. The pressure loss in B-100 was 5% at full load and 18:1compression ratio, however B-10 shows a loss of 1 to 2%. Hence 10% additive blend with mineral diesel can be recommended for fuelling diesel engines without any engine modification and adulteration.

Brake thermal efficiency

The variation in brake thermal efficiency with load by fuelling absolute *Madhuca indica* bio-additive, B-10, B-20, B-30, B-50, and mineral diesel was taken for analysis as presented in Fig-8. The brake thermal efficiency of mineral diesel was found highest from part load to full load and that of B-100 was found lowest because of higher viscosity, low calorific value and poor spray characterics. The brake thermal efficiency in B-100 was lowered by 5% at full load and 18:1 C.R; however B-10 was lowered by 1 to 2%. Hence 10% additive blend with mineral diesel can be recommended for fuelling diesel engines without any change in engine hardware and adulteration.

Specific fuel consumption

The variation of specific fuel consumption per kilowatt hour with load by fuelling absolute *Madhuca indica* bio-additive, B-10, B-20, B-30, B-50, and mineral diesel was taken for analysis as presented in Fig-9. The specific fuel consumption per kilowatt hour of mineral diesel increases at part load and decreases gradually as the load increases. The profile of mineral diesel was found above from part load to full load and that of B-100 was found below which was addressed to higher viscosity, low calorific value and poor atomisation character of *Madhuca indica* bio-additive. The specific fuel consumption per kilowatt hour for mineral diesel and B-10 were very close from part load to full load. Hence 10% additive blend with mineral diesel can be recommended diesel engines without any engine modification or adulteration.

Emission analysis

Emission analysis of B-10

The emission profiles of B-10 and mineral diesel were considered for comparison as presented in Fig-10. Emission of CO, CO_2, and free O_2 by fuelling B-10 was very close to that of mineral diesel. Hazardous emissions like HC and NO_x were considerably less (47%) than that of diesel. So 10% *Madhuca indica* bio-additive blend with mineral diesel can be recommended for fuelling diesel engines without any hardware modification or adulteration.

Emission analysis of B-20

The emission profiles of B-20 were compared with mineral diesel as displayed in Fig-11. Emission of CO, CO_2, and free O_2 was very close to that of mineral diesel. Hazardous emissions like HC and NO_x were considerably less

(45.45%) than that of diesel. So 20% *Madhuca indica* bio-additive blend with mineral diesel was considered slightly lower grade fuel package in comparison to 10% blending and can be recommended for fuelling diesel engines without any modification. However its higher viscosity than B-10 may cause clogging of fuel filter in long run i.e. more than 200 hours.

Emission analysis of B-30

The comparison of emission profiles of B-30 considering mineral diesel as baseline fuel was displayed in Fig-12 for analysis. Emission of CO, CO_2, and free O_2 was very close to that of mineral diesel. Hazardous emissions like HC and NO_x were considerably less (44.4%) than diesel. So 30% *Madhuca indica* bio-additive blend with mineral diesel was slightly lower quality fuel package in comparison to 10% and 20% blend because of higher viscosity, durability problem and poor injection characteristics.

Emission analysis of B-50

The emission profiles of B-50 were considered, referring mineral diesel as baseline fuel for analysis as presented in Fig-13. Emission of CO, CO_2, and free O_2 was very close to that of mineral diesel. Hazardous emissions like HC and NO_x were considerably less (34.34%) than diesel. So 50% *Madhuca indica* bio-additive blend with diesel was slightly inferior fuel package while comparing with 10%, 20% and 30% blend, because of higher viscosity, durability problem and poor atomising character.

Emission analysis of *Madhuca indica* (B-100)

The emission profiles of B-100 were considered for analysis referring mineral diesel as base line fuel as presented in Fig-14. Emission of CO, CO_2, and free O_2 was very close to mineral diesel. Hazardous emissions like HC and NO_x were considerably less (25.2%) than diesel. So absolute *Madhuca indica* bio additive was an inferior fuel package while comparing with 10%, 20%, 30% and 50% blend because of higher viscosity, durability, aging and poor injection quality through nozzle.

CONCLUSION

This study experimentally analyzed the characteristics of cold flow performance and exhaust emissions of *Madhuca indica* bio-additive in absolute and additive package with mineral diesel. The low temperature flow properties of *Madhuca indica* bio-additive was an imperative problem in fuelling as commercial fuel. Preheating up to 100^0C and subsequent cooling to atmospheric temperature mitigated the problem substantially. *Madhuca indica* bio-additive in 10% to 20% blend with mineral diesel improved cold flow properties and viscosity considerably, rendered proximal combustion to diesel without any modification in engine hardware or adulteration. The engine modification in utilising exhaust gas temperature for preheating may mitigate the problem substantially for fuelling higher additive blends of *Madhuca indica* bio-additive. However higher viscosity, poor cold flow properties, low calorific value, aging and poor injection quality of *Madhuca indica* bio-additive predominated its use as an absolute commercial fuel. The emission studies inferred contemporary CO, CO_2 and O_2 release when compared with mineral diesel. However HC and NO_x emissions were considerably lower than mineral diesel. Noise level was low without cracking sound as observed in fossil fuel combustion. The energy harnessed was sustainable clean and safe irrespective of poor cold flow properties and negligible power loss.

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Table-1 Pysicochemical properties

Property	Mineral diesel	DO	*Madhuca indica* bio-additive	ASTM D6751-02	DIN EN14214
Density@ 40^{0}C , kg/m^3	835	945	890	875-900	860-900
Kinematic viscosity @40^{0}C, cst	2.44	47.09	20.99	1.9-6	3.5-5.0
Flash point in ^{0}c	70	226	198	>130	>120
Fire point in ^{0}C	76	250	203	>65	>70
Cloud point ^{0}C	-10 to -15	14	12	Summer =4 Winter= -1	Summer=6 Winter=1
Pour point ^{0}C	-35 to -15	15	8		
Acid value, mg of KOH/gm of oil	NM	30	1.12	<0.8	<0.5
Calorific value(MJ/kg)	43	35	38.863	40 min	49 max
Saponification value	NM	191	130	.....	.....

Colour	Light brown	Slight greenish yellow	Dark yellow	.....	.....
Cetane number	47	NM	50	.....	.....
Iodine value	NM	65	60		
Diesel index	150	140	145	.....	.....

Table.2 Eengine specification

Engine	Kirloskar TV1
General details	4 stroke CI water cooled single cylinder computerised.
Bore x Stroke	87.5 mm x 110 mm
Compression ratio	17.5 : 1(varing from 16:1 to 18.1)
Displacement	661 cc
Power	3.5 kW
RPM	1500

Fig 1. *Madhuca indica* tree with different stages of maturity

Fig 2.Fuel filter clogging in 200 hours engine trial

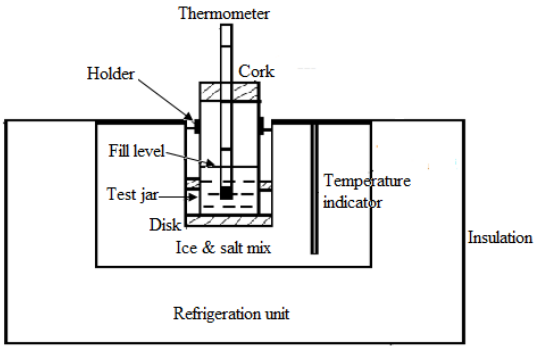

Fig.3ASTMD-2500 specified cloud point setup

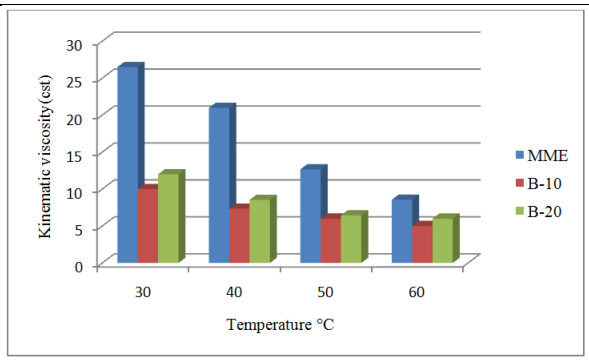

Fig.4 Variation of viscosity with temperature

Fig.5 Computerised engine equipped with equation solver

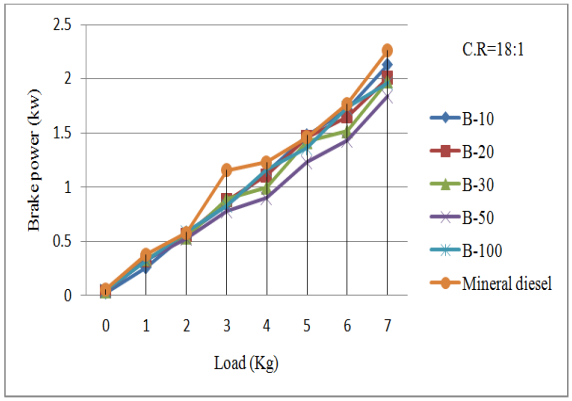

Fig.6 Variation of brake power with load

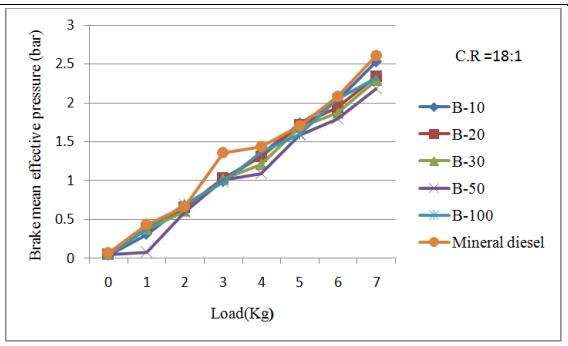

Fig.7 Variation of brake mean effective pressure with load

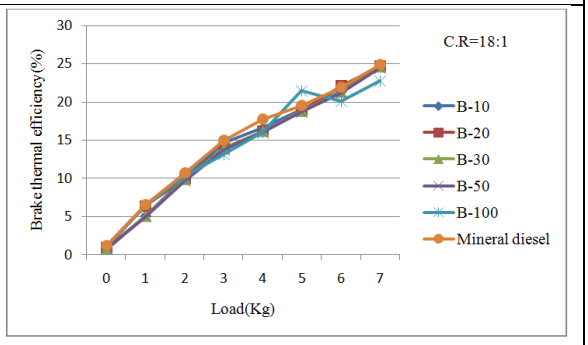

Fig.8 Variation of brake thermal efficiency with load

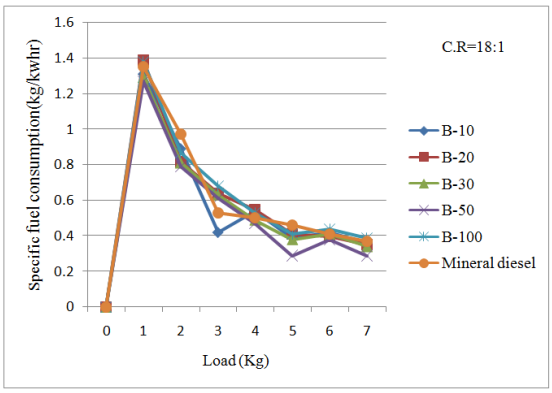

Fig.9 Variation of specific fuel consumption with load

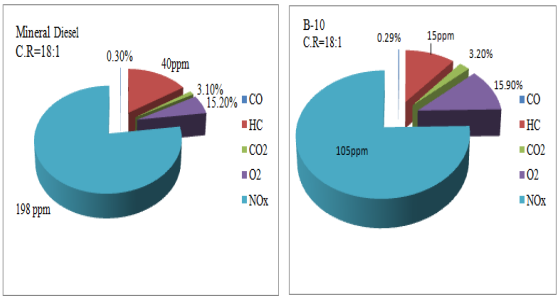

Fig.10 Emission profiles of mineral diesel and B10

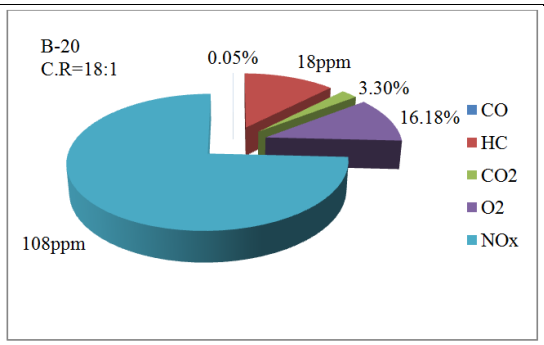

Fig.11 Emission profiles of B20

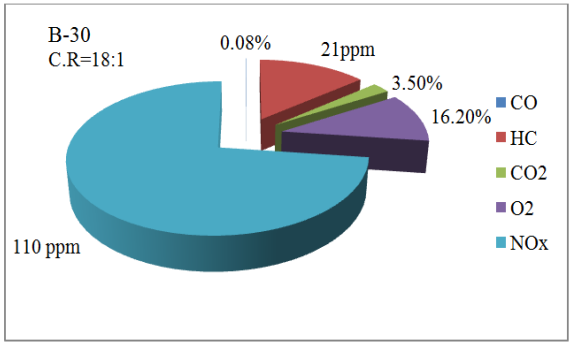

Fig.12 Emission profiles of B 30

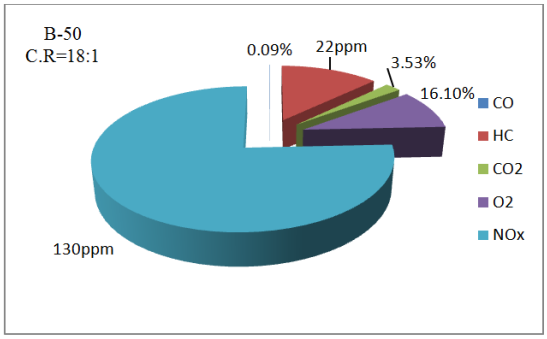

Fig.13 Emission profiles of B 50

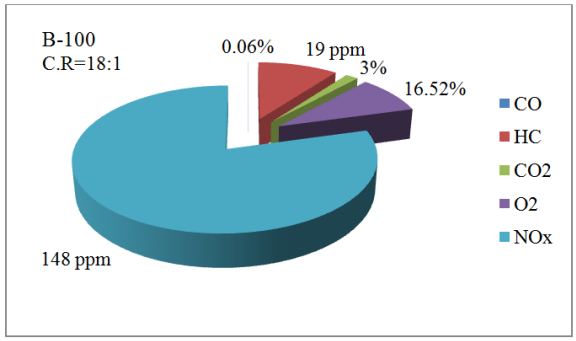

Fig.14 Emission profiles of B 100

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RESEARCH ARTICLE

Vietnamese High School Students' Perception of Self-Compassion

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ABSTRACT

Self-compassion is an adaptive way of relating to the self when considering personal inadequacies or difficult life circumstances(Neff & McGehee, 2010). This study aimed to investigate Vietnamese high school students' perception of Self-compassion . The sample was composed of 247 students (77 boys and 144 girls from grades 10, 11, and 12) of 16–18 ages attending three high schools in Ho Chi Minh City, Vietnam. They completed a 26-item the Self Compassion Scale (SCS), which Kindness subscale, Self-Judgment subscale, Common Humanity subscale, Isolation subscale, Mindfulness Subscale, and Over-Identification subscale. The most common Self-compassion among the students was found to be "Self-compassion " (M =3.04, SD = 1.01). The results also found that high school student's Self-compassion is good, with no significant male and female to the perception of Self-compassion . The present study investigated the Kindness subscale, Self-Judgment subscale, Common Humanity subscale, Isolation subscale, Mindfulness Subscale, and Over-Identification subscale among high school students from Ho Chi Minh City, Vietnam.

Keywords: Self-compassion , Students, High school.

INTRODUCTION

Self-compassion is defined as an attitude of kindness and understanding toward one's disappointments and struggles that include three interconnected, components: mindfulness, self-kindness, and common humanity (Neff, 2003). Research by Neff and McGehee (2010) found that Self-compassion was strongly associated with well-being among adolescents as well as adults, family, and cognitive factors were identified as predictors of individual differences in Self-compassion . Felder and Dimidjian (2016) suggested that Self-compassion warrants further attention in the study of the development, maintenance, and treatment of perinatal mood and anxiety disorders. According to Neff, Kirkpatrick and Rude (2007), they showed that mindfulness is related to psychologically adaptive

variables and that Self-compassion is a crucial attitudinal factor in the mindfulness–happiness relationship. Another research (López, and Schroevers (2018) found that Compassion for others was higher in women than in men, and in low educated individuals compared to higher educated individuals. In contrast, according to research Bluth and Blanton(2015), they found that Self-compassion was lower in low educated individuals, they found that older female adolescents had lower Self-compassion than either older male adolescents or early adolescents of either gender, and Self-compassion was associated significantly with all dimensions of emotional well-being with the exception of positive affect(Bluth & Blanton, 2015). Similar, Yarnell and Mullarkey (2015), they found that revealed males had slightly higher levels of Self-compassion than females, with a small ES observed (d = .18). Self-compassion was strongly associated with well-being among adolescents as well as adults(Neff & McGehee, 2010). However, they are not mentioned about student's perception of the Self-compassion Scale(Neff and McGehee, 2010) in measuring Self-compassion in Vietnamese high school students are still limited. We have been conducting studies designed to help fill this gap. Our research is conducted to empirically explore high school students' perception of Self-compassion in Ho Chi Minh City, Vietnam. The purpose of this study was to investigate the level of Self-compassion , essential needs during high school, and relationships between Self-compassion , essential needs, and characteristics of high school students.

MATERIALS AND METHODS

Measurement

Participants were asked to complete the following questionnaire: the Vietnamese versions of the Self-compassion Scale (SCS) for students based on the original(Neff, 2003). The SCS consists of six subscales: Kindness (K); Self-Judgment(SJ); Common Humanity (CH); Isolation(I); Mindfulness(M); and Over-Identification (OI). The 26 items of SCS were translated into Vietnamese by two bilingual researchers who were both familiar with the construct being assessed. For one of them, the first language was Vietnamese, and the other the first language was English. Forward and backward translation procedures were used. The same sequence of items was maintained in the Vietnamese translation of the index. All participants were instructed to read the questionnaire questions carefully and choose the responses that best described themselves. The SCS consists of 26 items measured on a 5-point Likert scale in which the 1 indicates a response of ‘almost never’, while the value of 5 corresponds to ‘almost always’. The internal consistency reliability (Cronbach’s alpha) estimate for this sample was fairly high at .60(Bowling, 2014). Alpha coefficients for each subscale were as follows: Kindness subscale: .88; Self-Judgment subscale:.88; Common Humanity subscale:.80; Isolation subscale:.85; Mindfulness Subscale:.85; and Over-Identification subscale:.88(Neff, 2003). One possible reason for the higher reliability of the subscale of the SCS scale could be the contextual differences; students responded to scale items according to their own understanding level. The Self-compassion Scale (SCS)

Participants

The convenience sampling method was used to recruit students who volunteered to help with the study and administer the survey. The survey instrument was distributed to 270 Vietnamese participants of tow high schools located in Ho Chi Minh City, Vietnam, of which 247 surveys were returned, for a 84% return rate, which exceeds the 30% response rate most researchers require for analysis(Dillman, 2000). The sample of this study was drawn from 247 respondents who completed the survey instrument. There were more girls 177(71.7%) than boys 70(28.3%) among the 247 Vietnamese participants who were surveyed. Of these, 54 (21.9%) were grade 10, 39(15.8%) were grade 11, and 154 (62.3%) were grade 12. Table 1 shows the distribution of participants.

RESULT

The mean score for the sample on the SCS (total score) was 3.04 (SD = 1.01). The mean score on the Kindness subscale was 2.88 (SD = 1.08). The mean score on the Sefl- Judgment subscale was 3.07(SD = .99). The mean score on the Common Humanity subscale was 3.09 (SD =.95). The mean score on the Isolation subscale was 3.06(SD=.95); The mean score on the Mindfulness subscale was 3.16(SD=1.04), and the mean Over- identification subscale was 2.98(SD=1.07). Table 2 presents descriptive statistics of dependent variables including Kindness subscale, Self-Judgment subscale, Common Humanity subscale, Isolation subscale, Mindfulness Subscale, and Over-Identification subscale. Table 2 of this study shows that the highest Self-compassion of the students is the Over-Identification subscale, including the following expressions: When I'm feeling down I try to approach my feelings with curiosity and openness (M =3.42, SD = 1.06). I try to see my failings as part of the human condition (M =3.40, SD = 1.03). I'm disapproving and judgmental about my own flaws and inadequacies (M = 3.37, SD = .99). The lowest Self-compassion of the students is the Kindness subscale, including the following expressions: I'm kind to myself when I'm experiencing suffering (M=2.55, SD=1.14). I'm tolerant of my own flaws and inadequacies (M= 2.69; SD=1.19). When I'm going through a very hard time, I give myself the caring and tenderness I need(M=3.26; SD=1.01). Table 3 shows the correlation coefficients between SK, SJ, CH, I, M, and O. The correlation coefficient for the SCS subscales ranged from 0.44 to 0.66. The correlation coefficient for the SCS subscales ranged from 0.44 to 0.66. The strongest correlation was found for the Over-Identification subscale(O) ($r = 0.66$, $p < 0.01$) and Common Humanity subscale (CH) ($r = 0.63$, $p < 0.01$). There was a weak positive correlation between Isolation subscale (I) ($r = 0.44$, $p < 0.01$).

DISCUSSION

The main goal of the present study was to explore high school students' perception of Self-compassion in Ho Chi Minh City, Vietnam. The main findings indicate that there was a positive relationship between six SCS subscales, especially, a positive and close relationship between Kindness subscale, Self-Judgment subscale, Common Humanity subscale, Isolation subscale, Mindfulness Subscale, and Over-Identification subscale. Results of research of our foundation show that the most higher of Self-compassion was When I'm feeling down I try to approach my feelings with curiosity and openness to mean (M=3.42; SD=1.06) most low Self-compassion was I'm kind to myself when I'm experiencing suffering (M=2.55; SD=1.14). It is a crucial conclusion for further upcoming studies regarding grow up Self-compassion among Vietnamese high school students that need to be conducted as soon as possible with high efficiency. This study reported that the level of students' Self-compassion has high, in men and women almost not the difference. That result was predictable, and it is consistent with what has been another research found that Compassion for others was higher in females than in males, and in low educated individuals compared to higher educated individuals(López and Schroevers ,2018). The results of this experiment are quite different from the opposite of (Neff & McGehee, 2010) Self-compassion was strongly associated with well-being among adolescents as well as adults. Although more research is needed to investigate whether high school students' perception of Self-compassion might be more pronounced in Vietnam because of its culture, the results suggest that studying high school students' perceptions may prove essential to understanding the Self-compassion in Vietname high school. This study has several limitations. The main limitation arises from the sampling process used. The sample was drawn from only one city of Ho Chi Minh City, Vietnam. The main limitation arises from the sampling process used. The sample was drawn from only one city of Ho Chi Minh City, Vietnam. The random selection of participants alleviates this concern to a significant degree but does not completely remedy that shortcoming. The second limitation is related to the sample and the self-reported measurements. This might bias the findings as well, and was cross-sectional research, which does not allow. Future studies should address these limitations by using a longitudinal design with data collected from high school and including more comparison communities.

CONCLUSION

The current study explored the understanding of the Self-compassion of high school students in Ho Chi Minh, Vietnam. It shows that the Self-compassion of high school students is good. Practical strategies to grow up Self-compassion in Vietnamese schools should be established with this result. It is the first research to investigate the understanding of Self-compassion by high school students in Ho Chi Minh City, Vietnam, to the best of authors' knowledge. In addition to the minimal research on this in Vietnam, Vietnamese school educators need to get a better understanding of their task.

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Table 1. An overview of survey participants

		N	%
Gender	Boy	70	28.3
	Girl	177	71.7
Grade	Grade 10	54	21.9
	Grade 11	39	15.8
	Grade 12	154	62.3
High School	An Lac high school	150	60.7
	Nguyen Van Linh high school	97	39.3

Table 2. The expression level of high school students' Self-compassion

	1		2		3		4		5		M	SD	Ranking
	n	%	n	%	n	%	n	%	n	%			
Kindness subscale													
I try to be understanding and patient towards those aspects of my personality I don't like.	14	5.7	116	47.0	73	29.6	36	14.6	8	3.2	2.63	.91	4
I'm kind to myself when I'm experiencing suffering	44	17.8	97	39.3	46	18.6	47	19.0	13	5.3	2.55	1.14	5
When I'm going through a very hard time, I give myself the caring and tenderness I need.	7	2.8	53	21.5	82	33.2	78	31.6	27	10.9	3.26	1.01	2
I'm tolerant of my own flaws and inadequacies.	45	18.2	70	28.3	69	27.9	42	17.0	21	8.5	2.69	1.19	3
I try to be loving towards myself when I'm feeling emotional pain.	20	8.1	52	21.1	55	22.3	81	32.8	39	15.8	**3.27**	**1.19**	**1**
Total											**2.88**	**1.08**	
Self-Judgment subscale													
When I see aspects of myself that I don't like, I get down on myself.			88	35.6	69	27.9	63	25.5	27	10.9	3.12	1.02	2
When times are really difficult, I tend to be tough on myself.	26	10.5	62	25.1	77	31.2	64	25.9	18	7.3	2.94	1.12	4
I can be a bit cold-hearted towards myself when I'm experiencing suffering.	0	0	103	41.7	77	31.2	52	21.1	15	6.1	2.91	.93	5
I'm disapproving and judgmental about my own flaws and inadequacies.	0	0	59	23.9	73	29.6	80	32.4	35	14.2	**3.37**	**.99**	**1**
I'm intolerant and impatient towards those aspects of my personality I don't like.	0	0	79	32.0	98	39.7	50	20.2	20	8.1	3.04	.92	3
Total											**3.07**	**.99**	
Common Humanity subscale													
When I feel inadequate in some way, I try to remind myself that feelings of inadequacy are shared by most people.	0	0	126	51.0	79	32.0	30	12.1	12	4.9	2.71	.86	4

I try to see my failings as part of the human condition .	0	0	60	24.3	70	28.3	76	30,8	41	16,6	**3.40**	**1.03**	**1**
When I'm down and out, I remind myself that there are lots of other people in the world feeling like I am.	0	0	92	37.2	71	28.7	60	24.3	24	9.7	3.06	1.00	3
When things are going badly for me, I see the difficulties as part of life that everyone goes through.	0	0	66	26.7	82	33,2	79	32,0	20	8,1	3.21	.93	2
Total											**3.09**	**.95**	
Isolation subscale													
When I fail at something that's important to me I tend to feel alone in my failure.	0	0	63	25.5	77	31.2	80	32,4	27	10,9	**3.29**	**.97**	**1**
When I think about my inadequacies it tends to make me feel more separate and cut off from the rest of the world.	0	0	107	43.3	56	22.7	63	25,5	21	8,5	2.99	1.02	3
When I'm feeling down I tend to feel like most other people are probably happier than I am.	0	0	74	30.0	95	38,5	58	23,5	20	8.1	3.10	.92	2
When I'm really struggling I tend to feel like other people must be having an easier time of it.	0	0	104	42.1	89	36.0	42	17.0	12	4.9	2.85	.87	4
Total											**3.06**	**.95**	
Mindfulness Subscale													
When something upsets me I try to keep my emotions in balance.	0	0	67	27.1	73	29,6	72	29.1	35	14.2	3.30	1.02	2
When I'm feeling down I try to approach my feelings with curiosity and openness.	0	0	64	25.9	59	23,9	80	32.4	44	17.8	**3.42**	**1.06**	1
When something painful happens I try to take a balanced view of the situation.	0	0	93	37.7	67	27.1	62	25.1	25	10.1	3.08	1.01	3
When I fail at something important to me I try to keep things in perspective.	23	9.3	67	27.1	91	36.8	50	20.2	16	6.5	2.87	1.05	4
Total											**3.16**	**1.04**	

Over-Identification subscale.													
When something upsets me I get carried away with my feelings.	16	6.5	80	32.4	81	32.8	56	22.7	14	5.7	2.89	1.01	2
When I'm feeling down I tend to obsess and fixate on everything that's wrong.	29	11,7	72	29.1	100	40.5	34	13.8	12	4.9	2.71	1.02	3
When something painful happens I tend to blow the incident out of proportion.	24	9.7	54	21.9	64	25.9	69	27.9	36	14.6	**3.16**	**1.20**	**1**
When I fail at something important to me I become consumed by feelings of inadequacy.	15	6,1	50	20.2	90	36.4	65	26.3	27	10.9	**3.16**	**1.06**	**1**
Total											**2.98**	**1.07**	
n: Number of participants; **%:** Percentage; **M:** Mean; **SD:** Standard deviation.													

Table 3: The correlations between groups of Self-compassion

	SK	SJ	CH	I	M	O
SK	1					
SJ	.52**	1				
CH	.53**	.65**	1			
I	.44**	.62**	.65**	1		
M	.48**	.51**	.54**	.61**	1	
O	.66**	.61**	.53**	.54**	.64**	1

**. Correlation is significant at the 0.01 level (2-tailed).

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RESEARCH ARTICLE

Waste Management of Eggshell using Vermicomposting Technique with *Eisenia foetida*: An Analysis through Changes in Physico-Chemical Parameters on Growth of Verm and Crop

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ABSTRACT

Worldwide consumption of hen eggs results in the production of large amounts of discarded egg waste particularly eggshells is an environmentally and economically challenging problem for it's proper disposal. It is produced from different sources like households, school and college hostels, hotels, fast-food restaurants, factories, etc. In the present study, the waste shells were utilized for the synthesis of highly active natural fertilizer by a combined composting-vermicomposting process with *Eisenia foetida*. Eggshell waste (ESw) treatment mixed with cow dung (CD) and soil (S) in the different ratio (1:1 (Control), 4:1 (S2), 3:2 (S3), 2:3 (S1), and 1:4 (S5), 100% ESw (Experimental)) as a substrate in experimental scale. The process was performed in three stages, including pre-composting, vermicomposting and plant growing which operate from 60-110 days in the pilot scale. The physicochemical analysis showed a significant increase in pH, high moisture content, suitable electro conductivity, and temperature. The rapid increase in physical growth as well as population growth of the earthworm seen after consumption of compost as nutrition. Vermiculture increased the no of worms in all treatments and the net weight and growth rate of worms in S1, S2 and Experimental were higher than other treatments. Therefore, the combined Composting-vermicomposting process can be used as an effective method for managing eggshell waste and CD. Germination of *Cicer arientinum*, *Cucurbita maxima* experimental proved the significant role of Eggshell waste (ESw) in sustainable plant growth.

Keywords: Egg shell waste (ESw), Vermicomposting, *Eisenia foetida,* Physico chemical analysis

INTRODUCTION

Organic waste contains materials that are biodegradable and originated from the living organism can be broken into carbon dioxide, methane, or simple organic molecules. Organic materials found in municipal solid waste include food, paper, wood, sewage sludge, and yard waste.The rapid population explosion and demand for chicken eggs produce of 2, 50,000tons annually all over the world, largely considered as useless and is discarded mostly because it contributes to pollution.China is the world's largest producer of discarded eggshell waste i.e. 24.8 billion kg, USA 5.6 billon kg & India 3.8 billion kg. (World FAO report 2015). It is mostly sent to the landfill with a high management cost. Sustainable management of huge amounts of eggshell waste is a major challenge. Eggshells waste materials from hatcheries homes, fast food restaurants, or industries (Phil and Zhihong, 2009; Amu et al., 2005) school and college hostel and hotels.

The eggshell contains a calcified shell and shell membranes including inner and outer members. Mac Neil (1997) developed a patent for separating eggshell membranes from the eggshell. The organic matter of eggshell or shell membranes contains proteins as major constituents with small amounts as carbohydrates and lipids (Burley and Vadehra, 1989). The composition of the eggshell is approximately 98.2, 0.9, 0.9% calcium carbonate, Magnesium, and phosphorus (phosphate) respectively (Romanoff et al., 1949). Shell membranes comprise approximately 10% collagen (Froning, 1998). Eggshells contain calcium and trace amounts of the microelements i.e. magnesium, boron, copper, iron, manganese, sulfur bi silicon, and zinc (King'ori, 2011). Eggshells are a good basis of calcium for plants as fertilizer. It plays a critical metabolic role in the removal of carbohydrates and neutralizes cell acids.

According to MOE (Ministry of Environment) India generates 62 million tons of waste every year out of which 450 grams per person per day. Municipal Solid Waste (MSW) generation ranges from 170 grams per person. As per the Ministry Of Housing and Urban Affairs Annual report for the year 2016-17 It is estimated that the total generation of solid waste is approximately 1,50,000 Tons per day. Out of the total 90% (1, 35,000 MT/day) is solid wastes, 20% (27, 00 MT/day) is processed and the remaining 80 %(10, 8000 MT/day) is going to dump sites. In Odisha total waste generation 9, 92,800MTPA, 12% of India .In Bhubaneswar it is 400 Tons/day. A large amount of waste would cause problems in waste disposal if they were not reused or recycled (India govt. portal). One solution to reduce landfill of the organic wastes was bioconversion of waste into a usable product. The biological processes commonly used by waste management are composting and vermicomposting.

The term "vermicompost" originated from a Latin word "Verms" meaning "worms". Vermicomposting is the process consists mainly of worm cast products in addition to decayed organic matter (Ismail 2005; Devi and Prakash 2015). Vermicomposting is natural and economical biotechnology of bio-converting agro-industrial residues through the mutual action of earthworm, micro-organisms, and enzymes to stable compounds for safe disposal and biofertilizer production (Suthar and Gairola 2014; Bhat et al. 2015; Musyoka et al. 2019) Aristotle, the Greek philosopher, recorded early the significance of worm in the biological system, and called them "intestine of the earth". Charles Darwin, the father vermicomposting. Darwin's last book, distributed in 1881, "The Formation of Vegetable Mold through the Action of Worms", with Observations on worm Habits. Father of vermicomposting in India Sultan Ahmed Ismail working on recycling biodegradable waste into fertilizer using local varieties of earthworm. Due to degradation activity is considered as "farmer's friend" or "Nature's plowman".The application of chemical fertilizers over a period has resulted in poor soil health, a reduction in productivity, and an increase in incidences of pest and disease and environmental pollution. (Ansari, A. A, and S. A. Ismail. 2001). Earthworms play a tremendous role in the management of biosolid wastes and protect the environment from hazardous effects.

In recent decades, various organic wastes such as horticultural residues from processed potatoes (Edwards CA, Neuhauser, 1988); mushroom wastes (Tajbakhsh J, Abdoli MA, etal 2008); horse wastes (Edwards CA, Fletcher KE, 1988); pig wastes (Chan LPS, Griffiths DA.1988); sericulture wastes (Gunathilagraj K, Ravignanam T, 1996);

municipal sewage sludge (Mitchell MJ, Hornor SG, Abrams BI,1980); agricultural residues (Bansal S, Kapoor KK, 2000); weeds (Bansal S, Kapoor KK, 2000); cattle dung (Bansal S, Kapoor KK, 2000); industrial refuse such as paper wastes (Butt KR.1993); sludge from paper mills and dairy plants (Banu JR, Logakanthi S, Vijayalakshmi GS, 2001); domestic kitchen wastes (Jaya Nair, Vanja Sekiozoic, Martin Anda 2005); urban residues and animal wastes (Rajeev p. singh, p. singh,A.S.F. Arauju etal 2011); sugar industry press mud (p Sangwan, C.p.kaushik, v.k. Garg, 2010); Fish meal and eggshells flour (Nurhidayati, Usman Ali and Indiyah Murwani,2017) are used as fretilizer.

Earthworms are terrestrial invertebrates and there are about 3000 varieties of earthworms. They have been formally classified into main 3 types based on their lifestyles and burrowing habits such are Anecic for(Greek for " out of the earth")(Canadian nightcrawler), Endogeic (Greek for " within the earth"), Epigeic (Greek for "upon the earth"). Earthworms body is dark brown, almost red glistening and covered with delicate cuticle. Earthworms are generally absent or rare in soil with a very coarse texture and high clay content or soil with pH 4 (Gunathilagraj, 1996). Epigeic types of earthworms are usually used for vermicomposting. The best types of worms for vermicomposting are tiger worm (*Eisenia foetida*) and red worms (*Lumbricus rubellus*), *Eudrilus eugeniae* (African Night crawler). In this study, earthworm species of *Eisenia foetida* were used to assess vermicompost due to its high adaptability to a changeable environment.

MATERIALS AND METHODS

Collection of Materials

The present experimental work wasconducted on vermicomposting about 60-110days for the duration of November to March (2020) in the campus of CUTM, located at Jatni, Bhubaneswar, Odisha, India. The required materials soils, cow dung, and eggshell were collectedfrom the CUTM campus. The soil was collected from the garden area of the CUTM campus. Cow dung was collected in large-sized plastic bag from cattle shed of the CUTM campus. Eggshell was collected from the hostel's canteen area of the CUTM campus.

Drying, Crushing and sieving of collected materials

The collected cow dung, soil, and eggshell were allowed to dry up exposing to the bright sun light. The process of drying was monitored every day at regular intervals until dry by spreadingthe materials. The large pieces of cow dung and soil were chopped into small during the drying process. The dried materials were sieved by a sieve having a diameter of 3.5 mm before weighing and collected in bags for early decomposition.

Collection of earthworm

The earthworm species that were used in this study i.e. *Eisenia foetida* were collected from the vermicomposting unit CUTM campus Bhubaneswar, Odisha and maintained in Zoology Laboratory with a control environment.

Experimental set up for pre-composting

The completely dried and sieved cow dung, soil, and eggshell were brought into the weighing site for measuring through weighing balance (ACZET CY224) with an accuracy of 0.0001gfor preparing up experimental setups by weighing different proportions. All materials such as cow dung (C.D.), soil (S), eggshell waste (ESw.)were taken in 6 different rectangular containers with 6 proportions labeled as control (1:1), S1 (4:1), S2 (3:2), S3 (2:3), S4 (1:4), ESw (100%) as given in Table 1. All the proportions were kept into their respective labeled containers of 30 cm length, 18 cm breadth, and 13cm height. All proportions in the containers were mixed uniformely at the dry stage and then allowed to sprinkle water till it reach with good moisture content. The mixture is allowed for composting for a week. At least 5 gms of both dry and wet of each proportion were collected and kept for analysis of various physicochemical parameters. The chemical composition was determined by XRF (X-ray fluorescence)technique.

Introduction of Earthworm

The various proportion of mixture after composting was used to make vermibed by layering the compost with adding of slurry in each labeled containers and kept in a shaded area for initiating of the process vermicomposting. Four numbers of earthworm species *E.foetida* of various lengths from 7 cm to 11 cm were put into each container shown in Table.3.

Culture of Earthworm

After introducing of earthworm into containers the nutrition as slurry regularly fed with an interval of 1-2 days for its survival. Sprinkling of water was carried out at regular intervals in the preliminary stage of vermicomposting and in the due period of processing till the completion within a 5-6 days of the gap to maintain moisture regularly. Regular monitoring of the vermicomposting process was observed and recorded until the process was conducted for 60-90 days.

Measuring of physico-chemical parameter

Physico-chemical analysis of waste in all proportions were measured like pH, temperature, and moisture content during the vermicomposting with a time interval of every 15 days .Determination of pH was done by digital pH meter through microcontroller at a temperature of 26.4°C (CHEMI LINE TECHNOLOGIES. The electro-conductivity by a conductivity meter using 1:20 (w/v) (LABROTECHNIQES_MH-16), and moisture content was determined by calculating the difference of dry mixture from the wet mixture using dry-oven method using standard ASTM D1762-84 for each container. All the parameters have recorded an interval of 15 days of the entire process.

Process of Vermicomposting

It was noticed that in the favorable environment the earthworms were started to reproduce juveniles after consuming the waste to grow and survive. The earthworm *Eisenia foetid*a that the waste converted to vermicompost after releasing of vermicasts due to the physiological action of it. In the regular intervals of time sprinkling of water was carried out every day in the preliminary stage of vermicomposting and in the period of processing it was repeatedly done within a gap of 4-5 days to maintain moisture regularly. Regular monitoring of the vermicomposting process was observed and recorded until the process was conducted for 70-90 days.

Harvesting of Earthworm

In order to facilitate the separation of worms from vermicomposting, the moisture content in the compost is brought down by stopping the addition of water before maturation for 4-5 days that ensures drying of compost a migration of worms into vermiculture containers. The remaining worms can be removed by hand as shown in (Fig. 10) The mature compost is removed out from the containers, dried, and packed for use in plant growth.

Germination Study

Germination was estimated after sowing seeds of *Cicer arientinum and Cucurbita maxima* on each vermicompost proportions. For germination testing another 6 containers labelled as same C,S1,S2,S3,S4,E were taken with different sample of obtained vermicompost soil were seen in Fig.11&12.

RESULT AND DISCUSSION

Various proportions of eggshell were utilized in the present study by using a recycling technology vermicomposting for the production of enriched organic manure and faster the degradable rate of eggshell by consuming and passing through the gut of earthworm. The physicochemical variables like temperature, electro-conductivity, pH, and water holding capacity were easily controlled and indicated progress of vermicomposting is figured and tabulated by different graphs and tables. From the present experimental work it was observed that each proportion varies with their physicochemical variables studied and observed with respect to electro conductivity, pH, and moisture content.

The pH value of each proportion mixed with eggshell powder in this experiment was ranged from 6.8 to 7.6 where the eggshell mixture reduced the acidity of soil may due to the presence of rich calcium. It was shown in Table-4 and Fig.3 that adding of eggshell powder into the slightly acidic soil converted towards alkaline due to addition with cow dung during the process of composting and vermicomposting. These processes were also involved with combining action of microbes and earthworms changed the pH towards alkaline within 7.5 which was suitable for the survival of earthworm and plant growth (Nisha Jain,2016). The electro-conductivity was increased from the initial value during vermicomposting might have added salinity to each proportion due to composting of calcium carbonate of eggshell.

The water holding capacity was slowly increased due to the wriggling action of earthworm in the soil and each proportion was maintained with the proper amount of moisture as compared to the whole mass of eggshell proportion. The temperature range was recorded below 35^0 C in each proportion that indicated the survival and population growth of earthworm which aided the formation of vermicompost agreed with (Taiwo and Oso, 2004). Besides all proportions in the whole eggshell mixed with cow dung were shown the expected result with granular vermicompost. The result from all aspects was favored towards the small proportion of eggshell i.e. S1, S2 including control whereas in S3, S4, and in eggshell-100% shown slow processing for vermicomposting. This study was reflected that all the physicochemical parameters were favored for the process of population growth which enhanced the production of vermicompost.

The growth of *Cicer arientinum and Cucurbita maxima* plants was proceeded at 5^{th} day after germination and reached at a rate 18-32 cm and 11-20 cm per 20 days respectively through scaling. The maximum growth was observed in S2 in both plant case and found a correlate result with S1 as compared to control. Whereas in other proportion growth was occurred but less than S1 and S2 agreed with (Amu, O. O.,et.al. 2005)(Table.6&7)

CONCLUSION

This present study was observed and studied that the eggshell waste from various sources produced vermicompost by using the technique vermicomposting also aid in growth of the plants. The eggshell contains calcium, magnesium, phosphorous, potassium, and sodium were consumed by earthworm during vermicomposting produced nitrogenous rich nutrient manure for sustainable plant growth. The addition of fine eggshell waste into normal soil converts to enriched soil with high fertility composition determined by various physico-chemical parameters. The survivability, growth and increased inpopulation are the key indicating parameters of formationvermicompost that enhanced crop production more as compared to control. Vermicomposting is not only converted eggshell wastes into organic manure but also plays a vital role in enhancement of crop production with rich nutrient might be fulfilled the demand of global food availability.

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TABLE 1.The composition of experimental set ups

Sl.No.	Experimental set ups	Ratio	Net weight
01	Control	1:1	1 kg
02	Standardization 1 (S_1)	4:1	1 kg
03	Standardization 2 (S_2)	3:2	1 kg
04	Standardization 3 (S_3)	2:3	1 kg
05	Standardization 4 (S_4)	1:4	1 kg
06	Experimental	100 % ESw	1 kg

TABLE 2. Measurement of Physico-chemical parameter during vermicomposting

Sl no	Observation	Electroconductivity			pH			Moisture content			Temperature ^{0}c	
	Experimental set ups	Initial	Final	Difference	Initial	Final	Difference	Initial	Final	Difference	Initial	Final
1	Control	0.715	0.937	0.224	6.8	7.2	0.4	0.389	0.487	0.98	33.5	31
2	S_1	0.251	0.549	0.298	6.71	7.23	0.52	0.163	0.286	0.123	30.8	29.7
3	S_2	0.364	0.713	0.349	6.93	7.26	0.33	0.170	0.295	0.125	30.5	28.9
4	S_3	0.440	0.964	0.524	6.86	7.29	0.43	0.292	0.330	0.038	30.7	29.2
5	S_4	0.745	0.984	0.239	7.48	7.27	0.21	0.444	0.495	0.051	31.3	29.9
6	Experimental	0.342	0.530	0.188	7.66	7.44	0.22	0.453	0.497	0.44	32.1	30.7

TABLE 3. The survival of Earthworm in various Experimental set ups

Sl no.	Experimental set ups	Initial no. of earthworm	Survival of earthworm in 1st15 days	Survival of earthworm in 2nd 15 days	Survival of earthworm in 3rd 15 days	Survival of earthworm in 4th 15 days
1	Control	4	Survival	Survival	Survival	Survival
2	S_1	4	Survival	Survival	Survival	Survival
3	S_2	4	Survival	Survival	Survival	Survival
4	S_3	4	1 Death	Survival	Survival	Survival
5	S_4	4	1 Death	Survival	Survival	Survival
6	Experimental	4	Survival	Survival	Survival	Survival

TABLE 4. The growth of Earthworm in various Experimental set ups through Mean ± SD

Sl no	Experimental set ups	Length in cm		Diameter in cm		Perimeter in cm	
		Mean	SD	Mean	SD	Mean	SD
1	Control	3.2	0	0.2	0.141	0.6	0.346
2	S_1	2.3	0.476	0.2	0.081	0.575	0.221
3	S_2	2.225	0.917	0.275	0.206	1.6	1.334
4	S_3	2.475	0.485	0.2	0.115	0.65	0.191
5	S_4	2.925	0.877	0.225	0.095	0.75	0.311
6	Experimental	5.525	1.463	0.575	0.051	1.975	0.377

TABLE 5. The population growth of Earthworm in various experimental set ups

Sl no	Experimental set up	Initial No. of Earthworm	Survival of earthworm in 15 days	Survival of earthworm in 30 days	Survival of earthworm in 45 days	Survival of earthworm in 60 days
1	Control	4	6	11	16	25
2	S_1	4	5	8	12	20
3	S_2	4	3	4	9	14
4	S_3	4	3	5	6	11
5	S_4	4	3	4	6	8
6	Experimental	4	4	6	12	16

TABLE 6. Germination duration & Plant growth of *Cicer arientinum*

Sl no	Experimental set ups samples	Germination duration	Plant growth length in cm observation from 0-20 days			
			0-5 days	05-10 days	10-15 days	15-20 days
1	Control	2 days	1.7	5	13.5	15
2	S_1	2 days	2.4	5.9	14.3	18.2
3	S_2	2 days	2.1	4	15.1	19.8
4	S_3	3 days	1.7	4.8	9.8	11.2
5	S_4	3 days	1	3.2	5.2	9.3
6	Experimental	3days	1	3	4.6	6

TABLE 7. Germination duration & Plant growth of *Cucurbita maxima*

Sl no	Experimental set up samples	Germination duration	Plant growth length in cm observation from 0-20 days			
			0-5 days	5-10 days	10-15 days	15-20 days
1	Control	2 days	3.2	7	16	27
2	S_1	2 days	3.8	8.2	18.5	29
3	S_2	2 days	4.2	8.8	19.7	32.5
4	S_3	3 days	3.6	7.4	18.2	26.5
5	S_4	3 days	2.8	6	15	23.4
6	Experimental	3 days	2	4.5	10.6	18.5

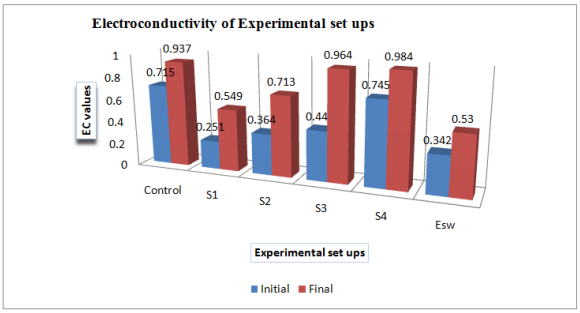

Fig. 1 Graphical representation of EC during vermicomposting

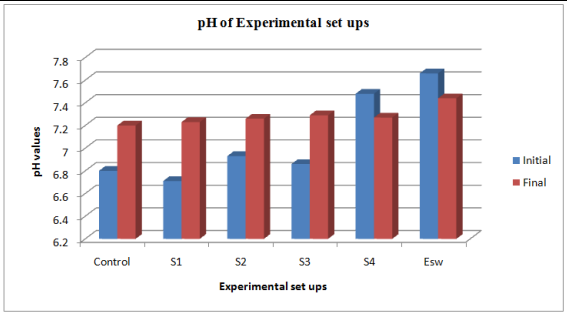

Fig. 2 Graphical representation of pH during vermicomposting

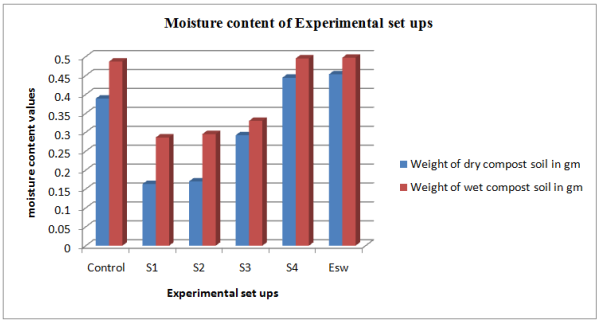

Fig. 3 Graphical representation of MC during vermicomposting

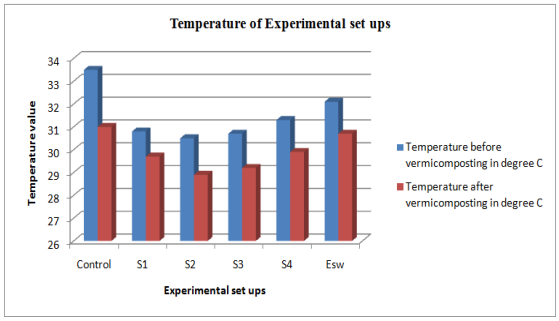

Fig.4 Graphical representation of Temperature during vermicomposting

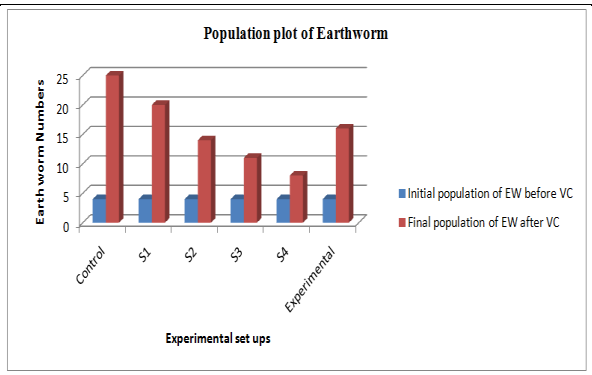

Fig. 5 Graphical representation of population growth

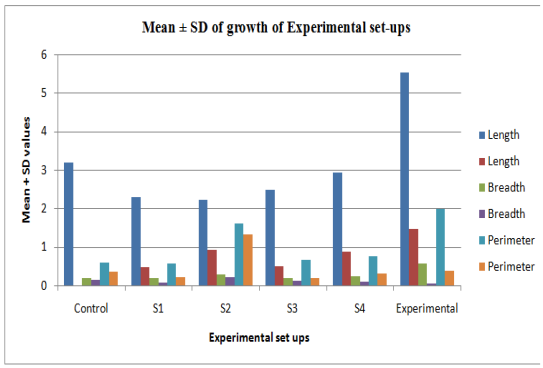

Fig. 6 Graphical representation of Growth of Earthworm

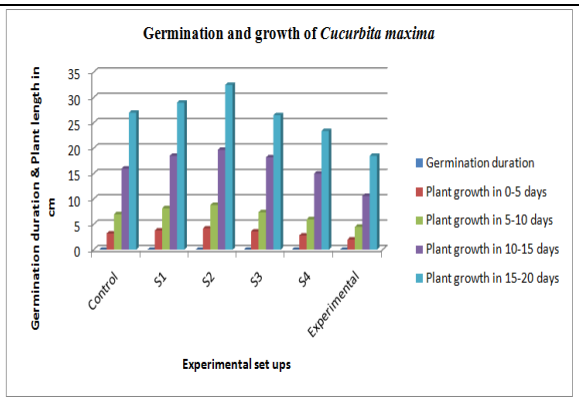

Fig 7. Graphical representation of germination and growth of *Cucurbita maxima*

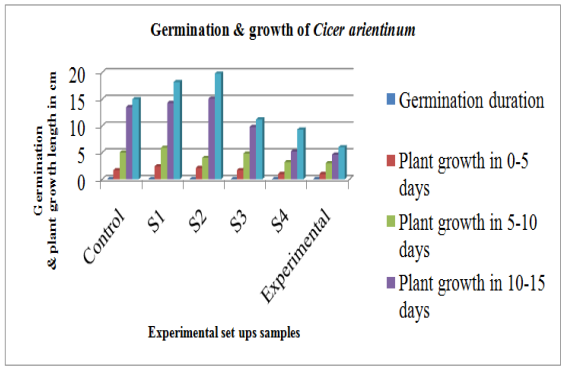

Fig 8. Graphical representation of germination and growth of *Cicer arientinum*

Fig 9. Earth worm at initial stage of Vermicomposting

Fig.10 Growth of earth worm and changes in structure during Vermicomposting

Fig 11. Germination and Plant growth of *Cicer arientinum*

Fig 12. Germination and growth of *Cucurbita maxima*

N.B: (EC-Electro-conductivity, ESw-Eggshell waste, EW-Earthworm, MC-Moisture content)

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RESEARCH ARTICLE

Molecular Identification of Actinomycetes with Effectual Antibacterials from the Sediments of Pichavaram Mangrove Forest, South India, by Sequencing the High G+C Content Genomic DNA

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ABSTRACT

The actinomycetes PMA2 and PMA6 were morphologically identified with Gram's staining method and they were identified morphologically as both are Gram^{+ve} organisms. For molecular identification, the genomic DNA was isolated and amplified the 16S rRNA gene with a set of primers 785F (5'- GGA TTA GAT ACC CTG GTA -3') & 907R (5'- CCG TCA ATT CMT TTR AGR TT-3'). The amplified products were confirmed by Agarose gel electrophoresis and the size of the products were determined and they were 1.6 Kb & 1.5 Kb. The PCR amplified products were sequenced, aligned for similarity search with BLAST and submitted the sequence in GenBank database. The accession numbers are MH183395 and MH183396. Phylogenetic tree was constructed and the actinomycete PMA2 was identified as *Streptomyces rubrolavendulae* strain BAP2 and the actinomycete PMA6 was identified as *Streptomyces alfalfa* strain VBMA6.

Keywords: Actinomycete, Genomic DNA, PCR amplification of 16S rRNA gene, Sequencing, Phylogenetic analysis.

INTRODUCTION

Microbial population functions significantly in the biotechnological and pharmaceutical industries by secreting enzymes, antibiotics and other valuable products. Microorganisms are capable of producing a variety of chemicals which are mostly bioactive secondary metabolites and they have high medicinal and industrial value [1]. Actinomycetes are Gram^{+ve}, filamentous, spore forming aerobic bacteria with high GC content in their DNA [2], exist in different places of nature and are rich in soil, fresh water and marine water [3]. Several unusual actinomycetes have been isolated from diverse locations like soil, marine environment and even from high salinity environments. Currently, researchers provide more attention to isolate actinomycetes from all ecological niches and developed several possible selection methods for their isolation [4].

Actinomycetes are the excellent sources of large bioactive compounds and have significant application in pharmaceutical and agricultural fields. *Streptomyces*, *Streptosporangium, Nocardiopsis* and *Microbacterium* are the major genera of actinomycetes were detected from diverse places including marine ecological niches. Several *Streptomyces* sp. was isolated and screened from diverse ecological niches for past numerous decades. Over 500 species of *Streptomyces* produces 70% to 80% of essential secondary metabolites [4]. Actinomycetes are the major sources of bioactive compounds which take part in therapeutic medicine, food, fermentation, textile and paper industries. The bioactive compounds also function as plant growth promoters, biocontrol agents, biopesticides and agro-active compounds [5].

Synthesis of secondary metabolite is a key feature of actinomycetes. Several antibiotics such as Streptomycin, Gentamycin, Rifamycin, Erythromycin and various anticancer drugs are synthesized from actinomycetes. Lot of commercially available compounds like antibiotics, antiparasitic, antifungal agents, anticancer and immunosuppressive agents are the products of actinomycetes [6]. The aim of the present investigation targets the molecular characterization of Actinomycetes which are having potent antibacterials and to identify the organisms through phylogenetic analysis.

MATERIALS AND METHODS

Morphological characterization

The actinomycete samples were characterized morphologically with Gram's staining method. Three days old cultures of actinomycetes PMA2 and PMA6 were stained differentially, the shape and cell wall components were analyzed with light microscopy [7,8].

Isolation of the Genomic DNA

Total genomic DNA was hauled out from the actinomycetes PMA2 and PMA6 by growing them in Starch Casein broth for 7 days [9]. They were confirmed by running in 1% Agarose gel electrophoresis. *Eco* RI digest of Lambda DNA was used as marker.

Amplification of the Genomic DNA

The genomic DNA of the organisms was amplified by using 16S universal primers. The primers used were 785F (5'-GGA TTA GAT ACC CTG GTA -3') and 907R (5'- CCG TCA ATT CMT TTR AGR TT-3') [10]. The amplification was performed in Bio-Rad (MyCycler, Bio-Rad, USA) Thermal cycler with 25.0µL reaction volume by adding 0.5 µL Template DNA, 1U *Taq* DNA polymerase, 1X *Taq* DNA polymerase buffer, 0.2mM dNTPs and 200 nM of each primer. The Thermal cycler was programmed for initial denaturation at 94^{o}C for 5 mins, denaturation upto 34 cycles with 94^{o}C for 45 seconds, annealing at 55^{o}C for 45 seconds, extension at 72^{o}C for 1 min and final extension at 72^{o}C for 5 mins [11]. Finally, the products of PCR amplification were detected in electrophoresis with 1% agarose gel. The molecular sizes of the PCR products were confirmed with the standard 1-Kb DNA ladder [3,12].

Phylogenetic analysis

Purification of the PCR amplified products of DNA was done by Macherey Nagel PCR clean-up kit followed by Automated DNA sequencing by terminating the DNA strands with dideoxy nucleotides [13]. Alignment of 16S rRNA gene sequence of actinomycetes PMA2 and PMA6 were performed using 16S rRNA gene sequence available in GenBank database using BLAST search tool [14,15]. The sequences were aligned with multiple sequence alignment and investigated phylogenetically by MEGA X software [12].

RESULTS

Morphological characterization

The actinomycetes PMA2 and PMA6 were characterized by Gram's staining method, both were Gram^{+ve} organisms and the shape was filamentous and rod, Fig – 1 shows the result of Gram's staining.

Isolation of the Genomic DNA

The genomic DNA was extracted from the actinomycetes, run in 1% agarose gel with *Eco* RI digest of Lambda DNA marker. DNA molecules of both PMA2 and PMA6 moved toward anodic end and they lied above 21Kb molecule. Fig – 2, shows the result of genomic DNA isolated from the actinomycete PMA2 (Lane 2) and the actinomycete PMA6 (Lane 3). Lane 1 consists of DNA marker (Lambda DNA - *Eco* RI digest).

Amplification of the Genomic DNA

The genomic DNA of the organisms was amplified by using 16S universal primers. The primers used were 785F (5'-GGA TTA GAT ACC CTG GTA -3') & 907R (5'- CCG TCA ATT CMT TTR AGR TT-3'). The amplification was performed in Bio-Rad (MyCycler, Bio-Rad, USA) Thermal cycler. The PCR amplified products were run in 1% agarose gel and both the products were identified between 1.5 Kb and 2.0 Kb DNA markers. Fig – 3, shows the result of PCR amplification, Lane 1 consists of DNA ladder (1Kb) and Lane 2 & 3 hold PCR amplified products of the isolate PMA2 and the isolate PMA6.

Phylogenetic analysis

The amplified products of 16S rRNA gene were sequenced with Automated DNA sequencing method. The sequencing result showed PMA2 consists of 1655 basepairs and PMA6 consists of 1549 basepairs. Alignment of 16S rRNA gene sequences of actinomycetes PMA2 and actinomycete PMA6 were performed using 16S rRNA sequence available in GenBank database using BLAST search tool. The actinomycetes PMA2 and PMA6 were highly similar with *Streptomyces* sp submitted already in Genbank databank. Based on 16S rRNA gene sequence, the actinomycetes PMA2 and PMA6 were identified as *Streptomyces rubrolavendulae* strain BAP2 and *Streptomyces alfalfae* strain VBMA6 respectively. The sequences were deposited in GenBank and the accession numbers were attained as MH183395 for *Streptomyces rubrolavendulae* and MH183396 for *Streptomyces alfalfa*.

Finally, Phylogenetic tree was constructed for each actinomycetes. Fig – 4, shows the similarity of actinomycete PMA2 (*Streptomyces rubrolavendulae* strain BAP2), it is more similar with *Streptomyces rubrolavendulae* strain MJM4426, complete genome followed by *Streptomyces rubrolavendulae* gene for 16S rRNA, partial sequence, strain NBRC 13683. The phylogenetic tree of actinomycete PMA6 (*Streptomyces alfalfae* stain VBMA6) shows that the organism is more similar with *Streptomyces alfalfae* strain ACCC40021 and Fig – 5 shows the similarity of the organism.

DISCUSSION

The requirement to find the new, effective and natural antimicrobials is very important to prevent antibiotic resistance by replacing the existing synthetic antimicrobial compounds [1]. Antibiotics are one of the bioactive compounds synthesized by actinomycetes. The genus *Streptomyces* is the major producer of antibiotics and 85% of

commercially available antibiotics are contributed by *Streptomyces*. The rest of 15% is contributed by other genera like *Actinomyces, Micromonospora, Actinomadura, Streptoverticillium* and *Thermo actinomycetes* [7]. Nowadays, sequencing bacterial genome became faster, though sequencing actinomycete genome is leisured till now. This condition may change because, the researchers are interested in natural products and the actinomycete genomes contain 30 gene clusters committed to secondary metabolite production [16]. The genome average G+C content has increment of 0.87% to 71.31% and the length of the genome also increases considerably at approximately 9% from 8.33 MB to 9.09 MB compared with first sequencing. It has offered influential proof that additionally sequenced DNA has G+C content at highest rate [17].

The actinomycetes PMA2 and PMA6 were initially characterized by their morphology. Gram's staining method revealed that both the organisms are Gram^{+ve} and the shape was filamentous and rod. Isolate AP-27 was observed under light microscopy and the organism was Gram^{+ve} spore forming organism. Spiral spore chain, open spore terminal and smooth surface were observed under Scanning Electron Microscopy (SEM) [6]. The total genomic DNA was extracted for amplification of 16S rRNA gene sequence. The genomic DNA was extracted fruitfully from S1A1 and S7A3 antagonistic bacterial isolates. The extraction of genomic DNA involves cell wall lysis, centrifugation for the removal of cell fragments and debris, precipitation of DNA and purification [8]. DNA purification kit – MB 527-50 pr (Himedia) was used for the isolation of genomic DNA from 12 active isolates. The PCR amplified products were investigated and all were about 1.5 Kb. The sequence was aligned using NCBI BLAST tool and all the isolates belong the genera *Streptomyces* [2].

The universal primers 785F and 907R were used for the amplification of 16S rRNA gene. The amplified products of actinomycete PMA2 and PMA6 were identified between 1.5 Kb and 2.0 Kb DNA markers. The amplified products were sequenced by automated DNA sequencer. The sequencing result showed 16S rRNA gene sequence of PMA2 consists of 1.6Kb and PMA6 consists of 1.5Kb. PCR amplified product of 16S rRNA gene from the isolate Ac1 was about 600 bp and sequencing result showed the length is only 403 bp [18]. 16S rDNA gene analysis is widely accepted and well suited method for several research laboratories. 16S rDNA is the part of ribosome and an essential component to synthesize proteins by all the prokaryotic organisms. 16S rDNA gene sequence is an appropriate molecular marker for the study of phylogenetic relationships [19]. The sequence of 16S rRNA gene has been confirmed to be a consistent genetic marker due to the presence in all bacteria. Even the function of that gene has not been altered eventually. This method can directly be functional on clinical samples and has established as an additional test in medical practice [20]. 16S rRNA gene sequence is unanimously exist in bacteria, extremely conserved and acts an excellent phylogenetic marker [21].

Alignment of 16S rRNA gene sequences of actinomycete PMA2 and actinomycete PMA6 were performed using 16S rRNA gene sequence available in GenBank database using BLAST search tool. The actinomycetes PMA2 and PMA6 were highly similar with *Streptomyces* sp submitted already in Genbank databank. The sequence alignment of 16S rRNA gene from isolate VUK-10 was done with all the available 16S rRNA gene sequence submitted already in Genbank database by BLAST. This sequence was highly similar up to 98% with the 16S rRNA gene sequence of *Pseudonocardia endophytica* [22]. The sequence of 16S rRNA gene of strain QI1 is more similar (99.3%) with *Natrinema gari*. Strain QI1 seems to be a new species within the genus *Natrinema*. Because, it is little bit dissimilar with *N. gari* in metabolic processes like Gelatin hydrolysis, Tween 80 hydrolysis and Arabinose hydrolysis [23]. The sequence of the isolate Ac1 was investigated by BLASTn tool, specified that the sequence belongs to *Streptomyces* sp and has given 98% sequence similarity [18].

The phylogenetic tree was constructed to find out the relationships of PMA2 and PMA6. They are highly similar with *Streptomyces rubrolavendulae* strain MJM4426 complete genome and *Streptomyces alfalfae* strain ACCC40021 respectively. Phylogenetic investigation of isolate AP-27 revealed that the organism is more similar to *Streptomyces griseus* [6]. Molecular identification of 57 bioactive isolates was done by 16S rRNA gene sequencing. All the isolates belong to Streptomycetaceae family [24].

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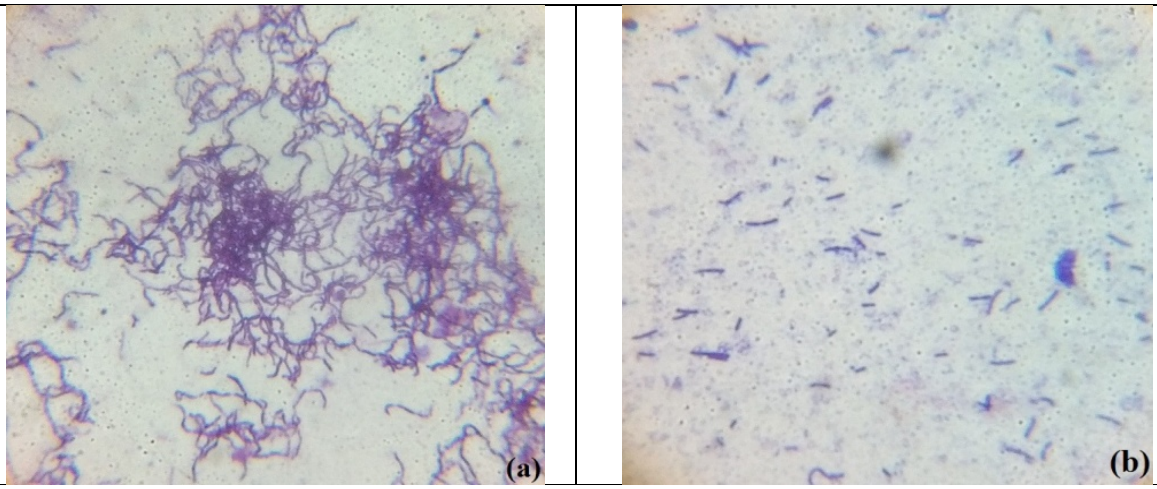

Fig – 1: Gram's staining of actinomycetes (a) PMA2 and (b) PMA6

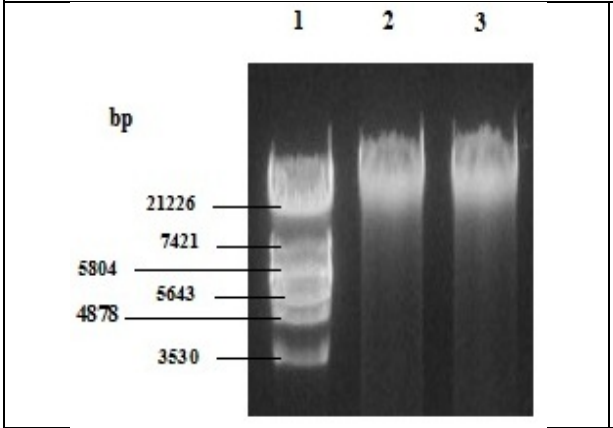

Fig – 2: Agarose Gel (1%) showing Lambda DNA / Eco RI Marker (Lane 1), Genomic DNA of actionmycete PMA2 (Lane 2) and actinomycete PMA6 (Lane 3)

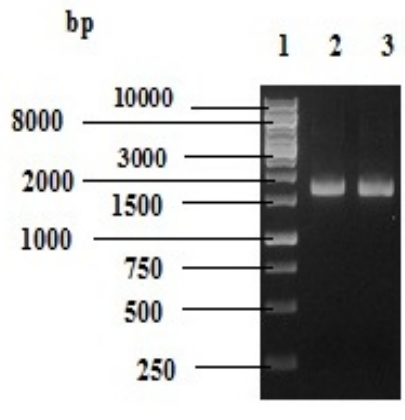

Fig – 3: Agarose Gel (1%) showing 1Kb DNA ladder (Lane 1), PCR Products of actionmycete PMA2 (Lane 2) and actinomycete PMA6 (Lane 3)

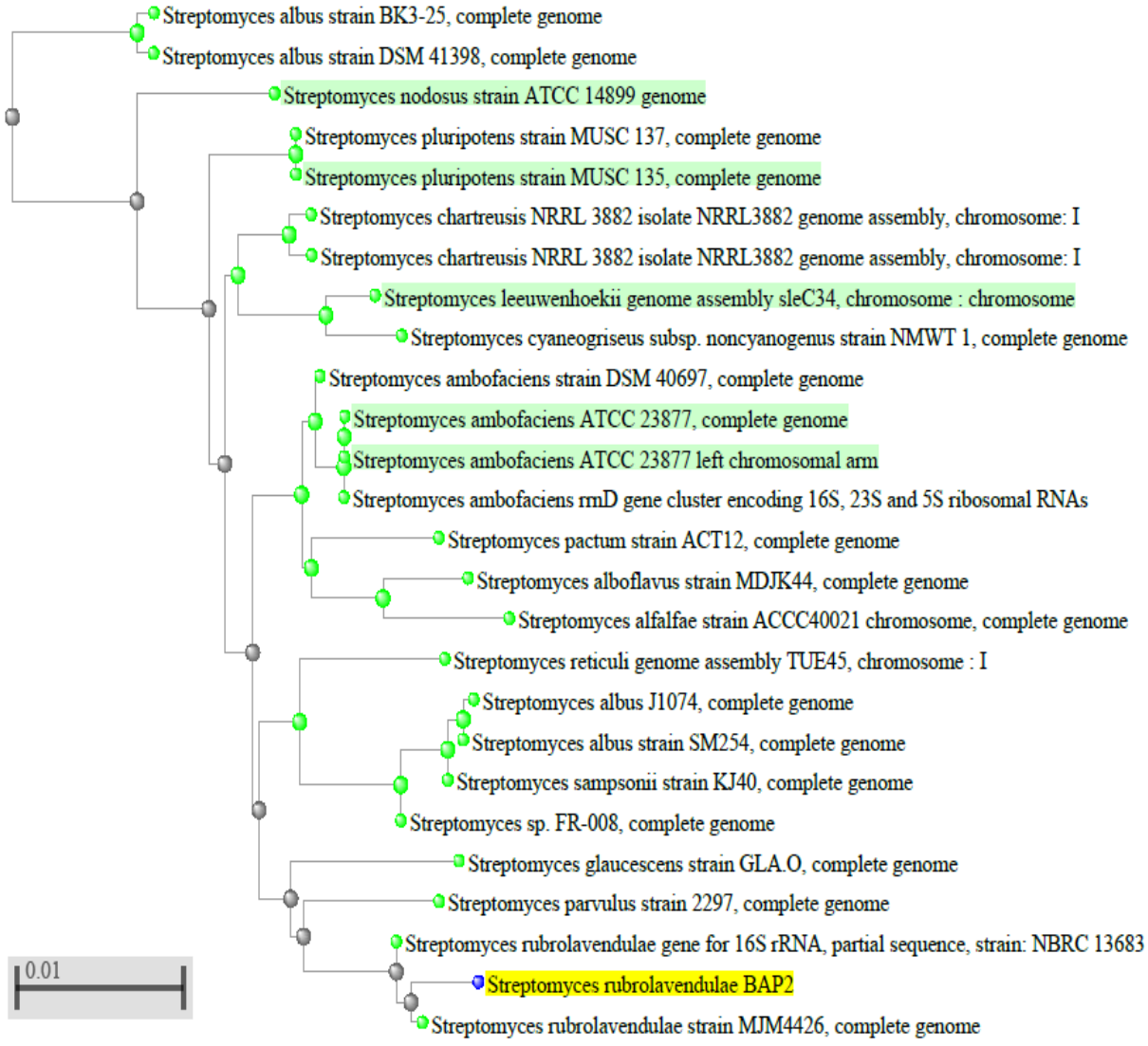

Fig – 4: Phylogenetic tree of PMA2 (*Streptomyces rubrolavendulae* strain BAP2) showing the similarity with *Streptomyces rubrolavendulae* strain MJM4426.

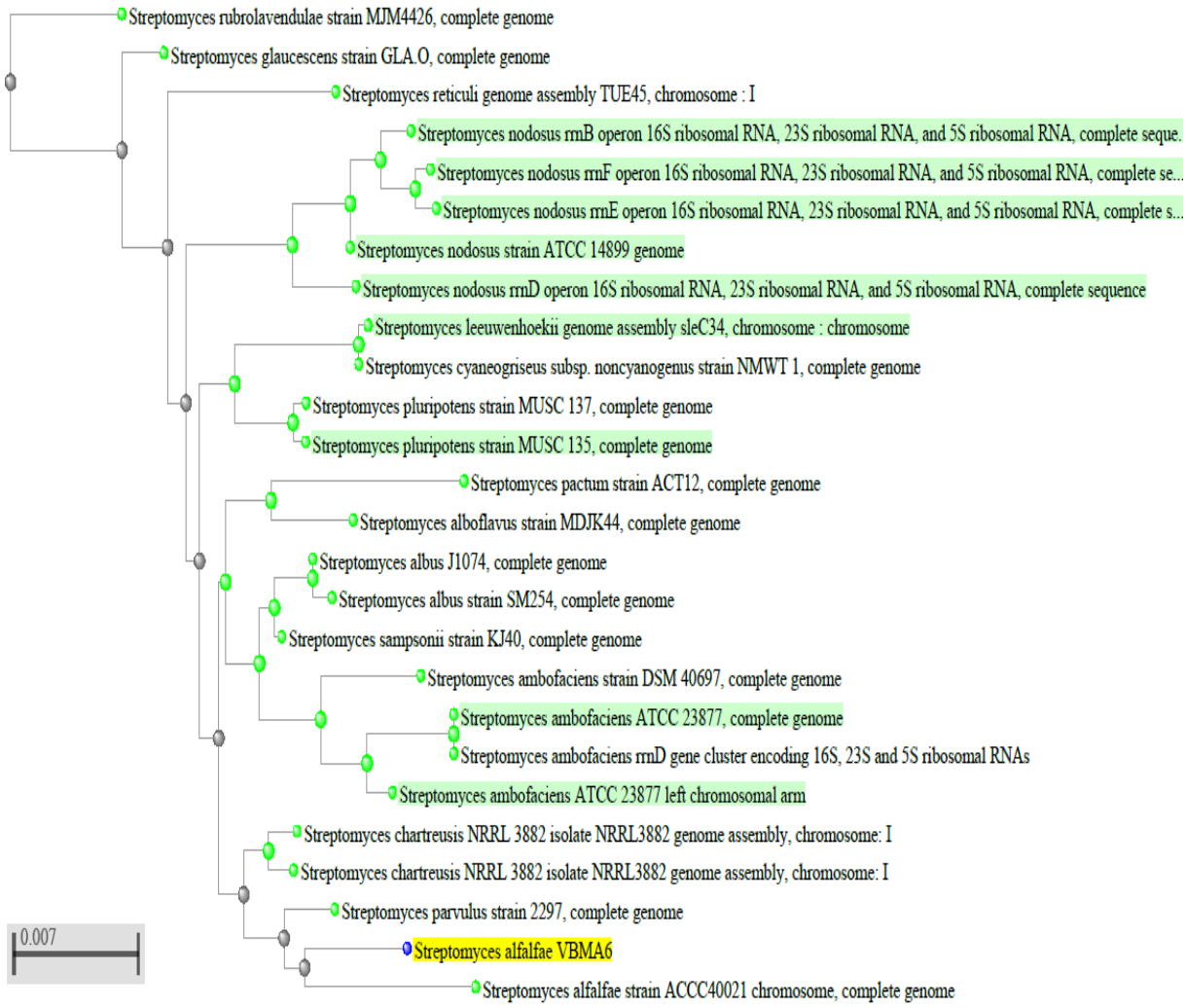

Fig – 5: Phylogenetic tree of PMA6 (*Streptomyces alfalfae* strain VBMA6) showing similarity with *Streptomyces alfalfae* strain ACCC40021

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RESEARCH ARTICLE

Production of Hydrogen Gas from Methanol using Dielectric Barrier Discharge Plasma Method

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ABSTRACT

Studies on hydrogen production from methanol using an indigenously designed dielectric barrier discharge (DBD) based plasma reactor have been reported. Different types of electrodes such as copper and feebly conducting waste water have been used in this experiment. Different experimental parameters such as electrical voltage and current were varied keeping the discharge gap constant and plasma discharge characteristics have been studied. The experiment shows the formation of filamentary types of micro-discharge at an interval of 0.32 μs with a discharge current of 0.4 ampere in case of copper electrode. But, in case of waste water electrode the discharge occurs at 0.036 ampere with an interval of 0.35 μs. Methanol degradation studies have been carried out in both the cases and rate of formation of hydrogen was found to be higher in case of waste water than copper case. Gas chromatographic studies confirm the production of high pure hydrogen gas from the plasma reactor upon the degradation of methanol. This experiment will highly effective in fulfilling the future energy demand by the generation of hydrogen energy.

Keywords: Dielectric barrier discharge, hydrogen generation, methanol, plasma, waste water

INTRODUCTION

Hydrogen is a renewable fuel and treated as a major source of alternative energy to meet the future energy demand in many petrochemical, automobile and chemical industry sectors. Future fuel cell market will be fully dependant on hydrogen as a primary fuel source. It can be formed from various sources of raw materials such as higher hydrocarbons, green house gases, refinery gas, wood, organic solid waste, sewages and alcohols derived from biomasses etc. Out of this, methanol has been used as the liquid fuel for conversion of the same by different technologies because of its high H_2 content, low-cost, easy storage and convenient transportation [1]. But,

thermodynamically methanol decomposition is not feasible as such in equilibrium condition (ΔH = +29.29 kJ/mol at 25 ^{o}C). Technologies such as steam reforming of natural gas and methanol based industrial processes are used for the production of H_2 at large scale. Recently, many methods have been introduced for the production of hydrogen such as stream reforming of methanol and natural gas [2-4], partial oxidation of methanol [5, 6], gasification of coal [7] and electrolysis of methanol water [8]. Gondal et al. achieved hydrogen yield of 20 mol % in 90 min by 90 min of laser exposure from methanol [9]. Similarly, Take et al. reported 97 mol % of hydrogen from methanol-water solution electrolysis [10]. But, all these processes need more energy. Thermal degradation of water at around 1500 ^{o}C has been practiced elsewhere. Because of this multi-dimensional features, plasma technologies have been employed in a number of applications, such as plasma spectra-chemistry, cutting, spraying, welding, etching or deposition of thin layers, catalysis ozone production, fuel gas cleaning and destruction of volatile organic compounds etc. [11-15].

Nonthermal and non equilibrium plasma techniques are becoming more popular because of the cost reduction and easy operational condition. It has capacity to make high temperature chemical reactions feasible at low ambient temperatures using much less input energy. Wide variety of chemical non-equilibrium conditions can easily be modified by external control, such as chemical input, pressure, electromagnetic field structure and discharge configuration. Using the non equilibrium plasma discharge, Wang et al. have studied the methanol (vapour form) conversion and have found that using stainless steel inner electrode, longer discharge zone and bigger discharge gap are beneficial for the reaction [16]. Methanol degradation study by Sarmiento et al. revealed that the effect of parameters such as voltage, frequency (1-6 kHz), roughness of electrode and flow rate etc. and found that less than 50 sccm flow rate enhanced the methanol degradation [17]. It has been observed that the roughness of the surface stabilizes the discharge. Tanaba et al. recently reported a dielectric barrier discharge (DBD) assisted decomposition of methanol into hydrogen [18]. Such DBD plasma methanol conversion is easier to be operated than the catalytic process, with an input power ranged from 0.2 W to 1 W. The maximum conversion of CH_3OH to H_2 is 90 %, which can be achieved in the absence or presence of water with the major by-products as CO and CO_2.

Nonthermal plasma processes for generation of hydrogen using ethanol, and ammonia have also been reported [15]. Similarly, corona discharges have also been used by taking methanol with 1.0 - 16.7 % water where ethylene glycol has been obtained as the major byproduct. Here –OH radical induces the oxidative dissociation of methanol forming $–CH_2OH$ radical. Liu et al. have made DFT calculations which favors formation of this radical in presence of water [19]. On the contrary, dielectric barrier discharges have been used for production of methanol or higher hydrocarbons from pure methane [20] and mixtures of methane with air, oxygen [21] and carbon dioxide [22]. Hydrogen generation efficiency from 0.02 – 1.5 mol H_2/kWh have been reported using different reactor configurations based on electrodes and dielectrics [15]. This paper presents a study on high pure hydrogen generation using indigenously developed simple DBD plasma set up based on both solid and liquid form of the electrodes. Attempt has been made to make a cost effective laboratory made DBD plasma reactor and to study its discharge characteristics to utilize alcohols for hydrogen generation. Using waste water as an electrode will help not only in production of hydrogen gas but also in producing clean water which can be used in agriculture. This process is described in Figure-1 pictorially.

EXPERIMENTAL

A schematic diagram of the plasma reactor is shown in the Figure-2. The reactor configuration was such that two hollow Pyrex glass tubes containing 2 mm dia copper electrodes were placed parallel to each other, with a discharge gap of 1.5 mm with a provision for easily placing and removing the same. In another set up, feebly conducting waste water (obtained from Puri sea beach, Odisha) was put in the glass tube which acts as one of the electrodes. A high voltage power source (CTP2000K plasma generator) capable of supplying bipolar sine wave output with 0-30 kV (peak-to peak) and AC frequency maximum up to 20 kHz was used. In this case, voltage was varied from 1.9 kV to 2.9 kV at 9 kHz frequency. Current – voltage waveforms as well as the charge vs. voltage of the discharge plots

obtained and discharge power was calculated. A digital oscilloscope (Tektronix make) was used for this purpose with a high voltage probe of 1000:1. The reactor has a provision to pass both liquid (organic and inorganic) and gases to the discharge zone. Analytical grade methanol was used for this work. Unlike in any other cases methanol was put in the reactor first and then voltage was applied. Provision was there to collect the gases and liquids after the discharge. The product gas generated in the process was collected using downward displacement of water and analyzed by a gas chromatograph GC-17A (Shimadzu) using a thermal conductivity detector (TCD) to detect hydrogen, carbon monoxide etc. Waste water was also used as electrode instead of copper and the different discharge characteristics were investigated. Decomposition study of methanol was carried out at different voltage (1.9 to 2.9kV) at 9 kHz keeping the residence time constant. Both yield and purity of hydrogen were determined. Based on the purity of the hydrogen further experiments were carried out at 2.4 kV and varying the time. Discharge characteristics using conducting waste water as an electrode medium was also studied and compared with that of the copper electrode.

RESULTS AND DISCUSSION

There are three phases of DBD discharge such as 1) breakdown, 2) quasi equilibrium stage and 3) non-equilibrium stage. The latter is the most effective for chemical reaction initiation due to presence of high energy electrons which can produce excited molecules and radicals. The experiment was carried out by designing a DBD plasma reactor whose real picture has been illustrated in Figure-3. The plasma discharge characteristics have been studied for methanol decomposition using both copper and conducting waste water as the electrodes. Discharge power was calculated from the discharge voltage and discharge current and it varied. In most of the experiments, 98 % pure hydrogen was produced. In case of copper electrode, it has been observed that with the application of voltage, filamentary types of micro-discharges are produced which can be observed in the form of streamer clusters in the current waveform depicted along with the voltage waveform of plasma discharge in Figure-4.

At different experimental parameters the degradation products were analyzed using gas chromatograms (Figure-5). It has been observed that on increasing the power, numbers of products (pertaining to the respective residence time) decreases from three to one. Above 0.51 W power, hydrogen with 98.99 % purity with residence time of 3.702 min. was produced which can be observed from the figure. In this case, other two other products with retention time at 0.117 min and 1.447 min were obtained below 0.4 W but the concentration of the same were very negligible (0.3%). Similar type of experiments was also carried out by taking waste water as one of the electrode. Product yield was calculated by using the following equation. Product yield (mol %) of hydrogen = 100 x [Product amount (mol)] / [Maximum amount of product evolved from methanol (mol)].

The mole of hydrogen was produced at different discharge power, keeping the time at 5 min. Initially at lower power, the rate of formation of hydrogen is slower than in case of higher discharge power. Similar trend has also been observed in case of waste water. Keeping the discharge power constant, methanol degradation study was carried out for 30 minutes and the % yield of hydrogen was determined. With increase in discharge time, the yield of hydrogen increases in case of both copper and waste water electrodes. But, it is higher in the case of waste water electrode assembly. Initially the rate of formation of hydrogen increases up to 15 minutes and then subsequently the rate decreases. Methanol conversion increased from 67 % to 88 % as the time increases from 5 minutes to 30 minutes. On increasing residence time, the rate of collision of methanol molecule with other ionic species like electron or radical increases which helps in the conversion of methanol to hydrogen and other products. Further, at constant input power and same dielectric material, discharge zone also plays a major role for the methanol conversion and hydrogen production. In the present study, discharge was characterized by multiple current pulse of a series of micro-discharge per half-cycle of the applied voltage. Discharge was continuous throughout the zone and continuous production of the gas was observed for 20 minutes. It was observed that after 20 minutes the rate of hydrogen production became slower and then increases with further increase in the power. During the discharge,

decomposition of methanolic intermediate species of the type OH, O, CO and CH_3O can be proposed. However, gas chromatographic study indicates the generation of CO and H_2 and confirmed with respective retention times. There is no carbon deposition found on the electrodes. Formation of hydroxyl radical (detected by hydrophilic test by on polyethylene) prevents the formation of carbonaceous species. It is to be noted that water as additional reactant, product like H_2, CO, CO_2, HCHO, ethylene glycol have been reported. DBD plasma reactors with different configuration with different types of dielectric material such as alumina and barium titanate have been discussed in terms of energy efficiency. In case of ceramic electrode material with high dielectric constant the energy efficiency increases. Unlike in any other process, feebly conducting waste water was used as an electrode and the energy efficiency was better than of the copper. The discharge characteristics of plasma discharge have been carried out by taking copper and compare with waste water as electrode. The value of input current was less for plasma discharge in case of waste water electrode as compared to copper electrode reactor. So, waste water electrode configuration is a better option for hydrogen generation with high purity and cost effective.

CONCLUSION

Using a very simple plasma reactor based on dielectric barrier discharge, hydrogen gas was generated. The DBD plasma reactor has been designed, developed and used for methanol degradation study. Two types of electrodes such as copper and waste water as outer electrode have been used keeping the inner electrode as Cu. Electrical characteristics were carried out to find out the input power of the DBD discharge. The micro-discharges are observed from the current waveform. The gas chromatographic study confirms the formation of hydrogen gas from the reactor with retention time 3.701 min. It has been seen that for waste water electrode assembly, more purer hydrogen with better yield has been produced making the system cost effective for practical use.

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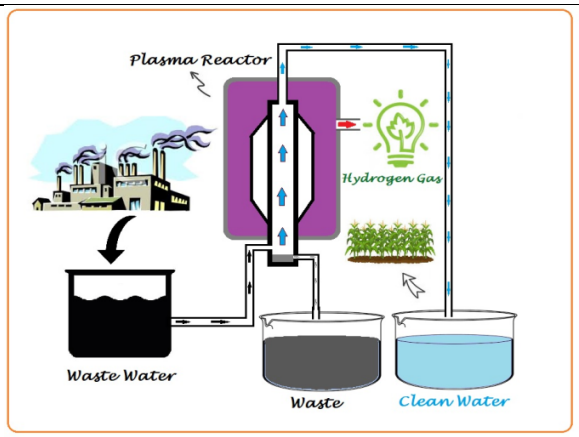

Figure-1: Treatment of wastewater for hydrogen production from methanol by a plasma reactor giving clean water for agriculture.

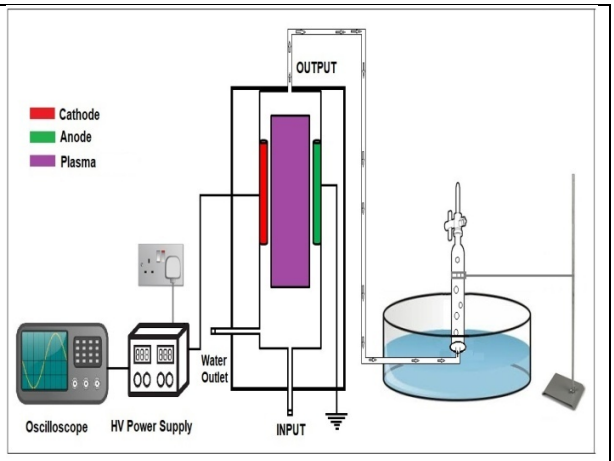

Figure-2: Schematic diagram of the experimental set up

Figure-3: Experimental set up constituting the plasma reactor indigenously developed in our laboratory for hydrogen generation.

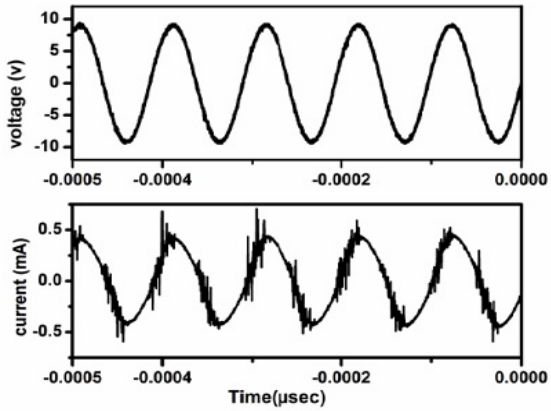

Figure-4: Voltage and current waveforms of methanol discharge by the DBD plasma reactor.

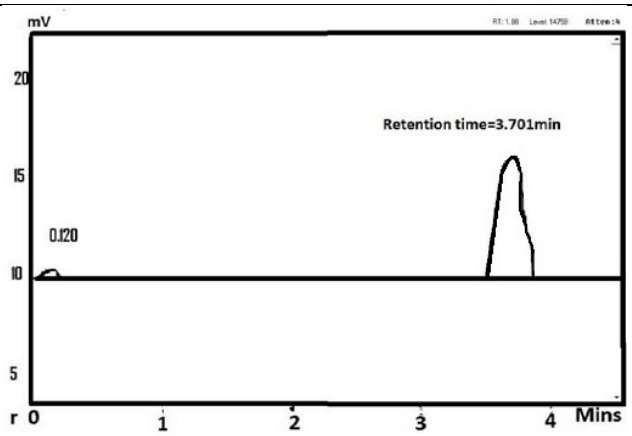

Figure-5: Typical gas chromatogram showing hydrogen generation at voltage 2.4 kV from the DBD plasma reactor.

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RESEARCH ARTICLE

Parametric Optimization of Biodiesel Synthesis and Property Modification of Biodiesel-Diesel Blends Using Palm Fatty Acid Distillate

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ABSTRACT

Biodiesel prepared from palm fatty acid distillate (PFAD) is blended with diesel fuel to improve certain properties. Initially, the reaction parameters for preparation of biodiesel from PFAD have been optimized. Identified optimized parameters are 5:1 molar ratio of alcohol to PFAD, 60^{0}C temperature, 8% (w/w) enzyme Novozyme 40013 (*Candida Antarctica*), an immobilized non specific lipase for 6 hrs of reaction. After that, blending of diesel fuel with PFAD and palm fatty acid distillate biodiesel (PFADB) has been done separately in definite proportions. PFADB was added in volume percentages of 20%, 40%, 60% and 80% in diesel fuel. The physico- chemical properties like density, kinematic viscosity, cetane number, acid value and flash point of different blends have been compared and good outcomes have been observed.

Keywords: Biodiesel, Palm fatty acid distillate, Diesel, *Candida Antarctica,* Blending

INTRODUCTION

Scarcity of non renewable fuels and continuous degradation of present environmental conditions demand replacement of non biodegradable fuels or use it by blending with biodegradable fuels [1,2]. Complete replacement of fossil fuels is next to impossible at this moment due to lack of alternative, environment friendly fuels. So only option goes to use it with mixture of biodegradable fuels. Among the biodegradable fuels, biodiesel is the best option due to its availability, ecofriendly nature, low toxic emission, less process hazards and minimization or no byproducts [3,4]. It can also significantly reduce exhaust emissions like hydrocarbons, carbon monoxide and

particulate matters at any ratios of blends with diesel fuel [5]. Biodiesel has been prepared from different cheap sources by many researchers6-9. Present author also synthesized biodiesel from various cheap sources [10-13]. The mixture of biodiesel and diesel is a good ways for overcoming the present burning problems. Currently, fuel energy content is one of the most important technical issues that indicate the use of biodiesel blend has a direct impact on the engine output power, its performance and emissions [14]. Blended biodiesel has also been prepared by many researchers for the investigation of properties compared to diesel fuel. Ali et al optimized the biodiesel-diesel blended fuel properties and engine performance using statistical analysis and response surface methods [15]. Silitonga et al analysed the properties of biodiesel diesel blends from edible and non-edible feedstock [16]. Wakil et al studied the physicochemical properties of biodiesel blending and predicted the properties of biodiesel blend through mathematical model [17]. Ali and Jaafar derived a relationship from physical properties of waste cooking oil / diesel blends and biodiesel fuels [18]. Oliveria and Da Silva identified a relationship between cetane number and calorific value of biodiesel from Tilapia visceral oil blends with mineral diesel [19]. Akhihiero et al studied the effect of blending ratio on the properties of sunflower biodiesel [20]. Analysis of properties of biodiesel-diesel blends have also been studied by many other researchers and academicians [21-25].

In the present research investigation, PFAD is used for biodiesel production and production parameters like molar ratio of alcohol to PFAD, temperature and enzyme concentration have been optimized for a definite time of reaction. After that PFADB was blended with diesel fuel apart from blending of PFAD and diesel fuel. The physic-chemical properties like density, kinematic viscosity, cetane number, acid value and flash point have been analysed and compared with different blending ratios. The blended fuel properties were characterized according to the American society for testing and materials blended fuel standard.

MATERIALS AND METHODS

Materials

PFAD was obtained from Emami Agrotech Ltd, Haldia, West Bengal. The enzymes used in the present study was Novozyme 40013, an immobilized non specific lipase from *Candida Antarctica* with ester synthesis activity of 10000 propyl laurate unit/g. The chemicals monoglycerides and diglycerides were purchased from Scientific and Laboratory Instrument Co., Kolkata. Diesel was collected from local market. Except otherwise specified all other chemicals were A.R. Grade.

Methods

Initially, PFAD was taken in an Erlenmeyer flask and heated up to 80^0C to drive off moisture by continuous stirring for about 1 h. After that, enzyme Novozyme 40013 was added and continuously stirred in the presence of alcohol maintaining proper reaction conditions. Alcohol was added in stepwise manner in an appropriate proportion using solvent hexane fitted with a water condenser and stirred by a magnetic stirrer at a specified temperature for 6 hours. Stepwise addition of methanol was allowed to minimize the deactivation of enzyme. During the reaction, continuous sampling and analysis were done for progress of reaction. The production of biodiesel was monitored by thin layer chromatographic (TLC) method and the typical yield of each reaction product was determined by column chromatography. TLC was done by spotting the lipid mixture on a silica-gel G plate (0.2 mm thick) using hexane-diethyl ether-acetic acid (90:10:1) as a developing solvent [26]. The composition of methyl esters was determined by column chromatography using silicic acid as an adsorbent and 160 mL of hexanediethyl ether: 99:1 as eluting solvent [27]. After completion of reaction, the enzyme was washed with hexane, dried and preserved for the next experiment. Values are reported as mean ± s.d., where n=3 (n=no of observations).

PFADB was added to diesel at low stirring rate. The mixture was stirred for 30 min and left to reach equilibrium before analysis. PFADB was added in volume percentages of 20%, 40%, 60% and 80%. In order to measure the

properties of the oil diesel fuels, the test methods were used as follows; density (ASTM D941), kinematic viscosity, cetane number, acid value and flash point.

RESULTS AND DISCUSSIONS

The composition of fatty acids, neutral glycerides and unsaponifiable matters present in PFAD was shown in Table 1. It was observed from Table 1 that PFAD contains higher amount of FFAs which mainly includes palmitic acid and oleic acid. Among other acids, linoleic acid shares maximum amount. Neutral glycerides namely triacylglycerols (TAG), diacylglycerols (DAG) and monoacylglycerols (MAG) are also an important part of PFAD which contributes 17.45±0.116% in the composition. PFAD contains little amount of unsaponifiable matters which mainly includes sterols and tocopherols. Before enzymatic hydrolysis, PFAD was thoroughly bleached to remove peroxides.

Optimization of molar ratio of MeOH to PFAD

The conversion of biodiesel production was analysed by varying the moles of alcohol with fixed amount of PFAD at temperature 60^{0}C using 8% (wt/wt) biocatalyst for 6 hours. Figure 1 shows that maximum conversion was achieved at 5:1 molar ratio of MeOH to PFAD.

Optimization of reaction temperature

Figure 2 shows the effect of reaction temperature for biodiesel production with 5:1 molar ratio of MeOH: PFAD, 6 hrs and 8% concentration of enzyme catalyst. Temperature variation analysed in our study ranges from 40 – 70^0 C and experiments showed that maximum biodiesel was obtained at a temperature of 60^{0}C. Increasing temperature did not enhance rather decrement occurred at higher temperature. It may be due to deactivation of enzyme at higher temperature.

Optimization of catalyst concentration

In our study, the effect of concentration of enzyme has been analysed by enhancing the amount of enzyme from 2-10% maintaining the same reaction parameters i.e. Temperature 60^{0}C, 5:1 molar ratio of MeOH: PFAD for 6 hrs which are indicated in Figure 3. It has been observed from the figure that maximum biodiesel was obtained with 8% (w/w) concentration of NS 40013.

Blending preparation and characterization

Two sets of blending were done for characterization purposes; one sample set was prepared for PFAD blended with diesel fuel and the other set was prepared for PFADB blended with diesel fuel as shown in Table 2. The blending is done based on volume percentage. Table 3 and 4 represent the comparative properties of blended fuels. Table 3 shows the properties of blended PFAD and diesel fuel and Table 4 shows the properties of blended PFADB and diesel fuel. The testing was repeated three times and was carefully recorded. In our study, five properties have been compared for blended fuels with respect to diesel fuel. It can be observed from the table that the fuel properties are greatly influenced by the concentration of the diesel fuel in the biodiesel and in the PFAD for the blended fuels. It is obvious that the blend fuels have different characteristics when being compared to mineral diesel. This because the component of the biodiesel contains free fatty acid (FFA) as the main constituent.

Density is a bulk and important fuel property of an oil or biodiesel if it is used as an engine fuel or playing roles in other applications. Table 3 and Table 4 show the results of density of PFAD/Diesel blends and PFADB/Diesel blends respectively against the percentage of blend. It is indicated that the density for both types of blend is increasing as the concentration of the PFAD and PFADB rises. Apart from that, it is also observed that the densities of PFAD/Diesel blends are again greater than that of the PFADB/Diesel blends in all proportions. This is due to the higher molecular mass of PFAD than that of PFADB. PFAD carried more free fatty acids with triglycerides which is heavier than the fatty acid methyl esters (FAME) with lower density and other advantageous fuel properties. Fuel

with higher density may be the cause of lessen life span of an engine and has immediate effect on the CI engine. So PFADB/Diesel blends are better choice than PFAD/Diesel blends with respect to density. Viscosity is an important property of fuel. Fuel with high viscosity can lead to poor atomization causing incomplete combustion and resulting in fuel injector blockage. So high viscous fuel has a long-term effect in damaging the engine. It is observed from Table 3 and 4 that the viscosity of both types of blends increases with the increasing amount of PFAD/PFADB in the blends. As the viscosity of diesel fuel is low and the viscosity of P100 or B100 is too high, so increasing amount of diesel fuel in the blends decreases the viscosity of blends. It is also observed that the viscosity of PFAD/Diesel blends is relatively higher than that of the PFADB/Diesel blends as they are approaching their pure form as neat PFAD/P100 and neat PFADB/B100 respectively. So transesterification process decreases the viscosity of the product which helps to use it as fuel. Although P20 is acceptable to be used in a diesel engine with regard to viscosity but it is not comparable with the properties of B20 making P20 an inferior fuel. It is highly recommended for PFAD to convert to PFADB so that the important fuel properties such as viscosity and density can be greatly improved.

Cetane number of a diesel engine fuel is the indication of its ignition characteristics. Higher the cetane number, better it is in its ignition properties. Fuels with lower cetane number have longer ignition delays, requiring more time for the fuel combustion process to be completed. Hence, higher speed diesel engines operate more effectively with higher cetane number fuels. Cetane number also affects a number of engine performance parameters like combustion, stability, drivability, white smoke, noise and emissions of CO and HC. It has been observed from Table 3 and 4 that B100 with cetane number 98 may be considered as ideal fuel for diesel engine. However, PFADB/Diesel fuel blends are also good as diesel engine fuel with regard to cetane number as B20 has higher cetane number (76.4) than diesel fuel (71.2). PFAD/Diesel blends have lower cetane number with the least proportion of PFAD (cetane number of P20 is 67.4 compared to 71.2 as diesel fuel). So biodiesel/diesel blends may be considered as a good diesel engine fuel for the present depleting nature of non renewable fuels.

Acid value is also an important characteristic of fuel. Higher acid value of fuel may corrode automotive parts and these limits protect vehicle engines and fuel tanks. Table 3 and 4 represent the acid value of diesel fuel, biodiesel and blends. B100 is ideal substitute for as diesel engine fuel with regard to diesel fuel due to its limiting acid value. PFAD/Diesel blends are not considered as diesel fuel due to very high acid value. So PFADB or PFADB/Diesel blends are a good option as substitute of diesel fuel for running C.I. engines with long term benefits. Flash point of a fuel is another important property which indicates volatility and flammability of a fuel being stored under ambient conditions. It is established that fuels with flash point lower than 130 °C is difficult to handle. Hence, fuel with high flash point is desirable not only to ensure safe storage but also beneficial in terms of fuel atomization and complete burning. Table 3 and 4 show the relationships between flash point and percentage of blend for the two types of blends used in this study. It has been observed that diesel fuel has low flash point (63^0C) due to which it is highly flammable. But blends of PFADB/diesel carry higher flash point e.g. the flash point of B20 is 93. Increasing the ratio of PFADB in blends enhances the flash point and ultimately B100 has a flash point of 187^0C making it the most stable and safe to handle fuel. Pure PFAD (P100) has much lower flash point than B100 measured as 147°C.

CONCLUSION

Palm fatty acid distillate has been identified as a cheap raw material for the production of biodiesel. Optimum parameters like molar ratio of alcohol to palm fatty acid distillate, temperature of reaction and concentration of biocatalyst have been analyzed for a definite time period of reaction. Palm fatty acid distillate biodiesel has been blended with diesel fuel in different ratios for studying the properties like density, kinematic viscosity, cetane number, acid value and flash point of blended fuels. A comparative properties has also been done with the blended of palm fatty acid distillate with diesel fuel. Our studies show that blended fuel can be safely used in different diesel engines without modification which can mitigate the environmental problems along with scarcity of non renewable fuels.

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Table 1: Analytical characteristics of PFAD

Component	Amount (% w/w)	Component	Amount (% w/w)	Component	Amount (% w/w)
FFA (Total)	79.25±1.313	Neutral glycerides	17.45±0.116	Unsaponifiable matters	3.1±0.003
Palmitic acid	49.15±0.256	TAG	37.26±0.136	Sterols	37.21±0.106
Oleic acid	35.67±0.157	DAG	43.06±0.179	Tocopherols	48.67±0.135
Linoleic acid	8.46±0.078	MAG	19.45±0.112	Hydrocarbon and others	13.26±0.121
Stearic acid	4.44±0.012				
Myristic acid	1.38±0.008				

Table 2: Blending composition of PFAD, PFABD and diesel fuel

Sample	PFAD	PFADB	Diesel fuel
*P 100	100	0	0
P 80	80	0	20
P 60	60	0	40
P 40	40	0	60
P 20	20	0	80
#B 100	0	100	0
B 80	0	80	20
B 60	0	60	40
B 40	0	40	60
B 20	0	20	80
P/B 0	0	0	100

*P-PFAD, #B-PFADB

Table 3: Properties of blended PFAD and diesel fuel

Properties	Density @ 15⁰C g/ml	Kinematic viscosity @ 40⁰C mm²/s	Cetane number	Acid value (mgKOH/g)	Flash point (⁰C)
Diesel fuel	0.834	2.29	71.2	0.22	63
P 100	0.889	4.79	51.6	79.25	147
P 80	0.879	4.31	55.1	64.68	135
P 60	0.868	3.81	59.7	48.57	117
P 40	0.857	3.33	63.8	32.87	101
P 20	0.847	2.83	67.4	18.21	82

Table 4: Properties of blended PFADB and diesel fuel.

Properties	Density @ 15⁰C g/ml	Kinematic viscosity @ 40⁰C mm²/s	Cetane number	Acid value (mgKOH/g)	Flash point (⁰C)
Diesel fuel	0.834	2.29	71.2	0.22	63
B 100	0.877	4.52	98	0.48	187
B 80	0.868	4.04	93.1	0.42	162
B 60	0.861	3.67	87.9	0.37	139
B 40	0.850	3.22	81.6	0.32	116
B 20	0.842	2.74	76.4	0.27	93

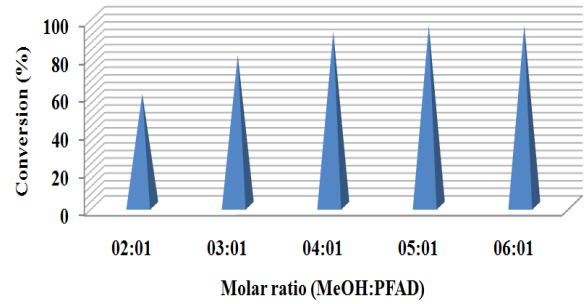

Figure 1: Analysis of molar ratio of MeOH to PFAD for biodiesel production [Temperature: 60⁰C, Time: 6 hrs and enzyme ns 40013: 8% (w/w)]

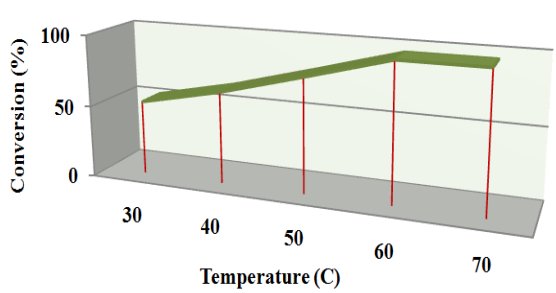

Figure 2: Analysis of temperature for biodiesel production [Molar ratio: 5:1 (MeOH:PFAD), Time: 6 hrs and enzyme NS 40013: 8% (w/w)]

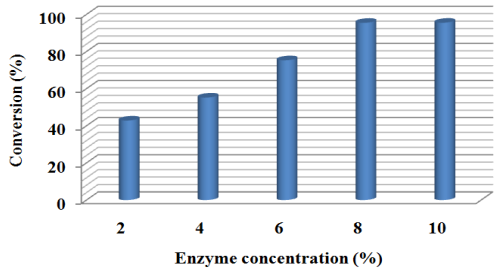

Figure 3: Analysis of enzyme concentration for biodiesel production [Molar ratio: 5:1 (MeOH:PFAD), Time: 6 hrs and temperature: 60⁰C]

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RESEARCH ARTICLE

Vegetational Analysis, Population Status and Girth Class Distribution of Gum Yielding Plants in Major Forest Patches of Sambalpur District, Odisha, India

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ABSTRACT

The study was carried out to analyze the species diversity of gum yielding plants in few forest patches of Sambalpur district, Odisha. Three forest sites of different size were selected for the study. A total of 45 individual tree species of 24 families were recorded, 14 different gum yielding plants were found in overall study sites. Out of three different sites, Chandlidungri was found to be more diverse in having a total of 26 number of different tree species and it was followed by Lakhmidungri and Daridungri. *Sterculia urens* exhibited maximum frequency in two sites and *Boswellia serata* exhibited in one site.The species area curve approached a normal depressive condition. The most diverse species was *Sterculia urens* for gum yielding plants. The most diverse site was Chandlidungri with2240 number of individuals of24different tree species. Since the study sites were diverse in different species of gum yielding plants, it is necessary to initiate the different approaches of conservation assessment that will improve the diversity ofgum yielding plants.

Keywords: Gum yielding plants, Girth Class, IVI, Forest Vegetation, Relative Density

INTRODUCTION

Forest is considered as the lungs of the planet earth; it continuously refining the air and maintain the percentage of the O_2 in atmosphere. Forest degradation, forest fire, deforestation and other anthropogenic effects are having reflective negative impacts on socio-ecological systems in the emerging economies across the tropics[1].A gross annual rate of deforestation of 0.05 % (2005–2013) in India has been reported by Reddy et al. in 2015 [2].Forest losses

were also noticed in country like, Indonesia, one of the highest rates of primary natural forest loss in the tropics, annually between 2001 and 2016 was reported [3]. Globally last year forest fire in amazon forest lost more than 1,600 square kilometers (619 square miles) of primary forest [4]. Periodical vegetational analysis is not properly done by the research communities now a days. Peoples are also less concern about the death of tree in their localities.

Vegetational analysis describes the diversity of plant communities[5] in forest site. Vegetation ecology includes the investigation of species composition and sociological interaction of species among communities [6].The gum yielding plant vegetation has got relatively little attention. The gum production is very much dependent on availability of gum yielding plants in sufficient number in appropriate girth size ***/*** age. Therefore, there arises a need to study the population structure of such plants in the natural forest. Population structure analysis can also indicate the regeneration potentialities of such plants. Few reports on natural regeneration of forest tree species belonging to sub-tropical broad-leaved forest in Shilong is available from the work of Barik et al. in 1992 and 1996 [7], [8]. Pandey et al. 2001 have also worked on degradation of *sal* forest in North-Eastern Uttar Pradesh [9]. Similar reports are also available on the tropical wet evergreen forest of Arunachal Pradesh[10] and forest of Kumaon Himalayas [11]. But all these works are based on economically valuable timber yielding plants. Reports available on the status of gum yielding plants in Indian forest is very meagre. Kala et al. 2013, while working on Pachmarhi Biosphere Reserve (Madhya Pradesh) have reported that population of some gum yielding plants like *Anogeissus latifolia* ,*Sterculia urens*, *Buchanania lanzan* were severely affected due to over exploitation[12].

The population status of the gum yielding plants has also been reported to be in a critical situation due to their poor germination capacity, non-availability of the conducive environmental conditions. *Sterculia urens*is one such gum yielding plant exhibiting very poor seed germination[13]. Micro-propagation of *Sterculia urens* has successfully done by Hussain et al. (2008) to increase the population of *Sterculia urens* and thereby increasing its availability for gum tapping and production[14].

STUDY SITE

The study sites are located in three forest patches close to Sambalpur University campus of Sambalpur, Odisha. Study was conducted in the three forest sites of Lakhmidungri, Daridungri and Chandlidungri (Fig.1.0) in the forest range Kamgam under Sambalpur south forest division, Sambalpur. The site Lakhmidungri located between 21^0 28' 24.02" N to 21^0 45'28.78"N and 83^0 52' 02.12" E to 83^0 77' 45.23"E and covering an area of 333.34 hectare with an average altitude of 170 m. from the sea level. The site Daridungri located between $22^0$25'48.66" N to $22^0$39'56.32"N and $83^0$52'54.07" E to $83^0$67'55.01"E and covering an area of 30.12 hectare with an average altitude of 55m from the sea level. The site Chandlidugri located between $21^0$29'15.78"N to $21^0$55'43.22"N and $83^0$55'44.63"E to $83^0$69'54.31"E and covering an area of 415.27 hectare with an average altitude of 220 m from the sea level. The forest type of the study sites is tropical dry deciduous and climate is tropical monsoonal.

METHODOLOGY

Population status and girth class distribution of gum yielding plants in the study site

The status of gum yielding plants in the respective study sites were ascertained by determining the frequency, density and girth class distribution of individual species in the three forest sites. For this purpose, in each site randomly 30 quadrates (size 10x10 M) were laid down on each site[15]. The different species of gum yielding plants on each site were recorded and the GBH (Girth at breast height in cm.) were measured. The frequencies of individual species were calculated by using formula given below.

$$\text{Frequency(F)} = \frac{Number\ of\ quadrates\ in\ which\ the\ species\ occurred}{Total\ number\ of\ quadrate\ studied}$$

Girth class analysis was done by measuring the GBH of all the plants above 10.5 cm. GBH and the girth class distribution was made following the classification of Rathanet al. (1982) (girth class A= 0-10.4 cm (Seedling), girth class B=10.5-31.4cm. (Sapling), girth class C= 31.5-65.4cm.(Pre-bole), girth class D= 66.5-100cm. (Bole), girth class E= 100.5-134.5cm. (Post-bole) and girth class F= 135-169cm. (Tree)) [16]. The density (number/ha) and the % density of individual species in respect to the total density of all the tree species was calculated by the following method of Mishra (1968)[17].

$$Density\ (D) = \frac{Total\ number\ of\ individual\ of\ the\ species}{Total\ number\ of\ quadrate\ used\ in\ sampling}$$

$$\text{Percent Density (\%D)} = \frac{Density\ of\ individual\ species}{Density\ of\ total\ number\ of\ species} X\ 100$$

Combined IVI calculation and Dominance-diversity curve of gum yielding plants

Frequency, Density and basal area of each gum yielding plant species in each and every study plot of all the sites were calculated and combined tofind out the average importance value index (IVI) in overall. Vegetation analysis is one of the best method to study the composition of species and structure of vegetation in an ecosystem and the IVI calculationis the best tool for this vegetation analysis [18]. Importance Value Index (IVI) is the sum total of relative frequency (RF), relative density (RD)and relativebasal area (RBA) for a plant species and it is calculated as below[19]. IVI $_{(sp.A)}$ = relative density of sp.A + relative frequency of sp.A + relative dominance of sp.A

$$RF = \frac{\text{Frequency of occurrence of the species}}{Total\ frequency\ of\ all\ the\ species} X\ 100$$

$$RD = \frac{Density\ of\ the\ species}{Total\ density\ of\ all\ species} X\ 100$$

$$RBA = \frac{Sum\ of\ basal\ area\ of\ all\ individuals\ of\ a\ species\ in\ the\ sample}{otal\ basal\ area\ of\ all\ the\ species\ in\ the\ sample}\ X\ 100$$

RESULT AND DISCUSSION

Understanding tree species diversity, composition, and structure is very important in assessing sustainability of forest, species conservation, and ecosystems management[20]. In overall study site, a total of 45 individual tree species of 24 families were recorded, out of total 14 different gum yielding plants were found(Table.1). The Vegetational analysis of the three study sites revealed that the sites differ in their species richness, density of plant species present and girth class distribution of species (Tab.2 and Fig.-2). The site Chandlidungri was found to be more diverse in having a total of 26 number of different tree species followed by Lakhmidungri with 20 numbers and Daridungri with 12 numbers of species. Simmilarly the tree species density was also found to be maximum in Candlidungri (ie. 2240 no. / ha.) followed by Lakhmidungri (1930 no./ha.) and Daridungri (1710 no./ha.). The percent density of individual tree species with respect to the total number of species present in the site when analyzed it was found that the percent density of *Sterculia urens* was highest (8.81%) in Lakhmidungri and that of *Boswellia serrata* and *Anogeissus latifolia* were found to be maximum ie. 13.45% and 7.02% in Daridungri site. In the Chandlidungri site the percent density of the individual tree species was found to be in moderate range. Frequency wise it was found that *Sterculia urens* exhibited maximum frequency(50%) in Lakhmidungri and Chandlidungri (40%) and *Boswelia serrata* in Daridungri (50%).

The girth class distribution (Tab.3 and Fig. 3.a, 3.b and 3.c) analysis indicated that the girth class distribution of the three species was different in three study sites. In the site Lakhmidungri *Sterculia urens* and *Anogeissus latifolia* were represented by four different girth classes ie. B,C,D and E whereas *Boswellia serrata* was represented by only three girth classes ie. C,D and E. *Sterculia urens* and *Boswellia serrata* were represented in the highest percent (32% and 69% respectively) in girth class D whereas *Anogeissus latifolia* was represented in its highest in its highest percent ie. (47%) in girth class C. In the site Daridungri *Sterculia urens* and *Anogeissus latifolia* were represented by three different girth classes ie. B,C and D whereas *Boswellia serrata* was represented by four girth classes ie. B,C,D and E. *Sterculia urens* was represented in the highest percent (48%) in girth class C, *Boswellia serrata* represented in the highest percent (37%) in girth class D whereas *Anogeissus latifolia* was represented in its highest percent ie. (44%) in girth class B.

In the site Chandlidungri *Sterculia urens* and *Anogeissus latifolia* were represented by four different girth classes ie. B,C,D and E whereas *Boswellia serrata* was represented by five girth classes ie. B,C,D,E and F. *Sterculia urens* was represented in the highest percent (31%) in girth class C, whereas *Boswellia serrata* and *Anogeissus latifolia* were represented in its highest percent ie. (31% and 37% respectively) in girth class B. In almost all the plants the girth class distribution was found in a pattern where the large girth classes were in low percentage. Thus, a class distribution curve of reverse 'J' shape was obtained, this pattern is very common to all kind of forests mainly in the logged-over forests where due to canopy openings the small trees emerges in the forest area [21]

Dominance-diversity curve

Data of table.4 and Figure 4 showed the dominance-diversity curve which is plotted between the IVI and sequence of species for gum yielding plants, it indicates a relationship among different gum yielding plant species showing importance value (IV) in overall 3study sites. This curve interprets that how Species dominance is relevant to the resource and suitable niche availability in a particular community[21]. For gum yielding trees, at the beginning, the curve started at highest point ie. 42.04 (*Sterculia urens*), then it moving consistent with a normal slope. The normal slope of the curve represents the steady growth of gum yielding plants, while continuous down movement of the curve representing the classes of small size of trees and it is the results of different anthropogenic disturbances (unsustainable logging).

CONCLUSION

This study showed that the study sites of Sambalpur district have diverse population of gum yielding plant species. A total of 14 gum producing trees of 9 different family were found at the sites. The most dominant gum yielding plant was *Sterculia urens* (Sterculiaceae) while the least available plat species was *Mangifera indica* (anacardiaceae). The most diverse site represented by Chandlidungri which comprise the maximum number of individuals (2240) and species (24). This paper concludes that a proper management and sustainable utilisation of gum yielding plants of the forest study site area may lead an establishment of the commercial gum production site in Sambalpur, Odisha.

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Table-1: Over all plant list with their family and local names in all the study sites:

No	Species	Family	Local name
1	*Semecarpus anacardium* L.f.	Anacardiaceae	Bhelua
2	*Alangium salviifolium*(L.f.) Wang	Alangiaceae	Aankel
3	*Holarrhena antidy centirca*(L.) Wall. ex A. DC.	Apocynaceae	Kure
4	*Bombax cebia*L.	Bombacaceae	Semel
5	*Boswellia serrata* Roxb	Burseraceae	Sale
6	*Bauhinia racemosa* Lam.	Caesalpiniaceae	Kuthel
7	*Bauhinia variegate* L.	Caesalpiniaceae	Kuthel
8	*Cassia fistula* L.	Caesalpiniaceae	Sunari
9	*Anogeissus latifolia*(DC.) Bedd.	Combretaceae	Dhaura
10	*Terminalia arjuna* (Roxb. Ex DC.) W.& A.	Combretaceae	Kaha
11	*Terminalia bellerica*(Gaertn.) Roxb.	Combretaceae	Bahada
12	*Terminalia chebula*Retz.	Combretaceae	Harida
13	*Terminalia tomentosa*(DC) W.& A.	Combretaceae	Sahaj
14	*Dillenia pentagyna* L.	Dilleniaceae	Rai
15	*Shorea robusta* Gaertn.f.	Dipterocarpaceae	Sal
16	*Diospyros melanoxylon* Roxb.	Ebenaceae	Kendu
17	*Cleistanthus collinus*(roxb.)Benth	Euphorbiaceae	Karla
18	*Emblica officinalis* Gaertn.	Euphorbiaceae	Aamla
19	*Mallotus philippensis*(Lam.) Muell.	Euphorbiaceae	Kath kamla
20	*Butea monosperma*(Lam.) Taub.	Fabaceae	palash
21	*Dalbergia sissoo* Roxb.	Fabaceae	Sissoo
22	*Erythrena suberosa* Roxb.	Fabaceae	Paldhua
23	*Pongamia pennata* L.	Fabaceae	Karanj
24	*Pterocarpus marsupium* Roxb.	Fabaceae	Bija
25	*Careya arborea* Roxb.	Lecythidaceae	Kumbhi
26	*Azadirachta indicia.* A.Juss.	Meliaceae	Neem
27	*Soymida febriguga* A. Juss.	Meliaceae	Ruhen
28	*Acacia catechu* (L.f.)	Mimosaceae	Khair
29	*Acacia leucophlea*Roxb.	Mimosaceae	guhira
30	*Acacia nilotica*(L.)Delile	Mimosaceae	Bamur
31	*Albizia lebbeck* Benth.	Mimosaceae	Shirish
32	*Ficus religiosa* L.	Moraceae	Pippal
33	*Syzygium cumini*L.	Myrtaceae	Jam
34	*Nyctathes arbor tistis* L.	Oleaceae	Gangasiuli
35	*Adina cordifolia*(Roxb.) Hook.f.ex.Bran	Rubiaceae	Haldu
36	*Canthium dicoccum*(Gaertn.) Merr.	Rubiaceae	Chikni

37	*Morindato mentosa* Heyne ex. Roth	Rubiaceae	Aachun
38	*Aegle marmelos*(L.) Corr.	Rutaceae	Bel
39	*Chloroxylons wietenia* DC.	Rutaceae	Bheru
40	*Schleicher aoleosa*(Lour.) Oken	Sapindaceae	Kusum
41	*Madhuca indica*Gmel.	Sapotaceae	Mahul
42	*Sterculia urens*Roxb.	Sterculiaceae	Girindel
43	*Symplocos racemosa*Roxb.	Symplocaceae	Tharro
44	*Lannea coromandelica* (Houtt) Merr.	Anacardiaceae	Joel
45	*Buchanania lanzan* Spreng.	Anacardiaceae	Chironji

Table-2: Species Richness, Frequency and Density of *Sterculia urens, Boswellia serrata*and *Anogeissus latifolia* with respect to other tree species in the individual study sites:

	F (%)	D (no/ha)	% D	Species Richness
Lakhmidungri				
Sterculia urens	50	170	8.81	
Boswellia serrata	20	20	1.04	
Anogeissus latifolia	30	90	4.66	
All species		1930		20
Daridungri				
Sterculia urens	20	20	1.17	
Boswellia serrata	50	210	13.45	
Anogeissus latifolia	20	120	7.02	
All species		1710		12
Chandlidungri				
Sterculia urens	40	120	5.36	
Boswellia serrata	30	60	2.68	
Anogeissus latifolia	30	50	2.23	
All species		2240		26

(F= Frequency, D= Density)

Table-3: Girth class distribution of *Sterculi aurens, Boswellia serrata* and *Anogeissus latifolia* with respect to other tree species in the study sites.

	A (%)	B (%)	C (%)	D (%)	E(%)	F (%)
LAKHMIDUNGRI						
All Species	-	**39**	**28**	**25**	**8**	**-**
Sterculia urens	-	19	28	32	21	-
Boswellia serrata	-	0	31	69	0	-
Anogeissuslatifolia	-	39	47	11	0	-
DARIDUNGRI						
All Species	-	**47**	**36**	**12**	**5**	**-**

Sterculia urens	-	18	48	34	0	-
Boswellia serrata	-	18	30	37	15	-
Anogeissuslatifolia	-	44	31	25	0	-
CHANDLIDUNGRI						
All Species	-	**48**	**23**	**16**	**12**	**1**
Sterculia urens	-	23	31	29	17	0
Boswellia serrata	-	31	27	20	13	9
Anogeissuslatifolia	-	37	31	23	9	0

A = (0-10.4 cm), B = (10.5-31.4 cm) ,C = (31.5-65.4 cm), D = (66.5-100 cm), E = (100.5-134.5 cm), F = (135-169 cm)

Table-4: Frequency(F), Density(D), Basal area(BA), Relative Frequency(RF), Relative Density(RD), Relative Basal Area(RBA) and Importance Value Index(IVA) of *gum* yielding plants with respect to other tree species in the combined study:

No	Species	Family	F	D/h	BA	RF (%)	RD (%)	RBA (%)	IVI
1	*Sterculia urens* Roxb.	Sterculiaceae	37	103	1.98	16.74	12.35	12.95	42.04
2	*Boswellia serrata* Roxb	Burseraceae	33	97	1.79	14.93	11.63	11.71	38.27
3	*Anogeissuslatifolia* (DC.) Bedd.	Combretaceae	26	86	1.29	11.76	10.31	8.44	30.51
4	*Shorearobusta*Gaertn.f.	Dipterocarpaceae	24	73	1.44	9.42	8.75	9.42	27.59
5	*Acacia nilotica* (L.)Delile	Mimosaceae	17	55	1.45	7.69	6.59	9.48	23.77
6	*Acacia catechu* (L.f.)	Mimosaceae	14	69	1.38	6.33	8.27	9.03	23.63
7	*Lanneacoro mandelica* (Houtt) Merr.	Anacardiaceae	14	67	1.12	6.33	8.03	7.33	21.69
8	*Butea monosperma* (Lam.) Taub.	Fabaceae	11	78	0.73	4.98	9.35	4.77	19.10
9	*Acacia leucopholea* Roxb.	Mimosaceae	10	37	1.21	4.52	4.44	7.91	16.88
10	*Aegle marmelos* (L.) Corr.	Rutaceae	7	47	0.86	3.17	5.64	5.62	14.43
11	*Albizia lebbeck* Benth.	Mimosaceae	11	29	0.91	4.98	3.48	5.95	14.41
12	*Azadirachta indicia*.A.Juss.	Meliaceae	9	33	0.54	4.07	4.02	3.53	11.63
13	*Buchanania lanzan* Spreng.	Anacardiaceae	3	33	0.37	1.36	3.96	2.42	7.73
14	*Mangiferaindica* L.	Anacardiaceae	5	13	0.22	2.26	1.56	1.44	5.26

Figure:1 Map viewing location of all three study sites ie. Lakhmidungri, Daridungri and Chandlidungri

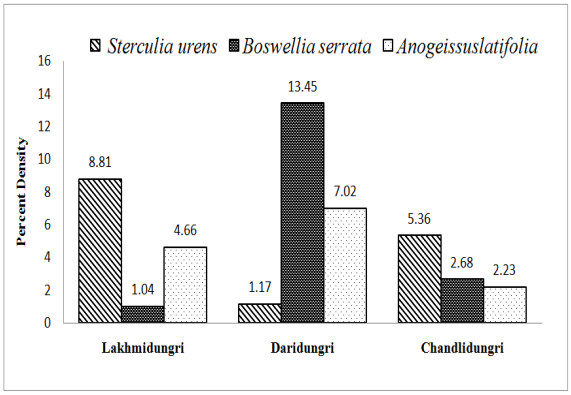

Figure -2: Percent density of *Sterculia urens*, *Boswellia serrata* and *Anogeissuslatifolia*in the different study sites.

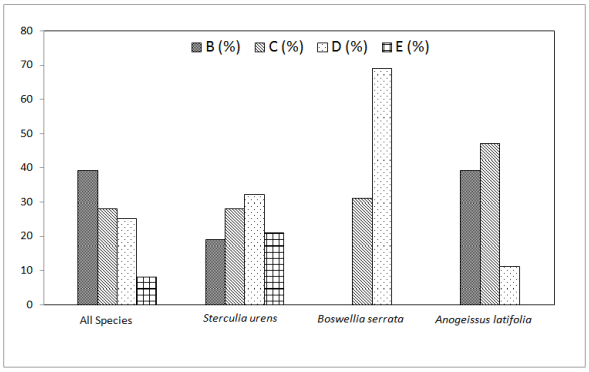

Figure- 3.a: Girth class distribution of *Sterculia urens*, *Boswellia serrata* and *Anogeissuslatifolia* with respect to other tree species in the study sites Lakhmidungri

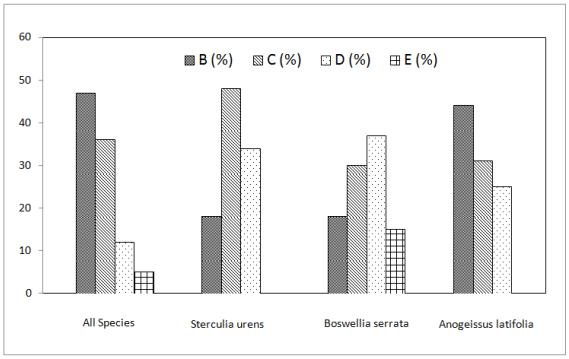

Figure- 3.b: Girth class distribution of *Sterculia urens*, *Boswellia serrata* and *Anogeissuslatifolia* with respect to other tree species in the study sites Daridungri

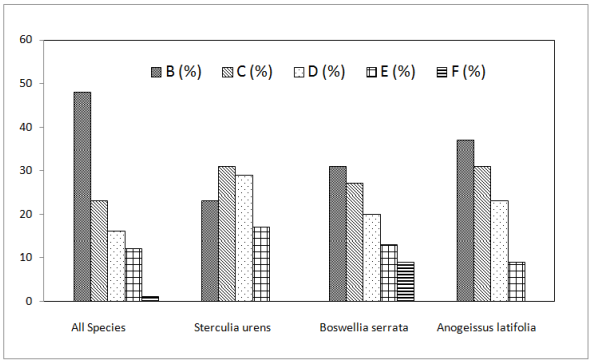

Figure- 3.c: Girth class distribution of *Sterculia urens*, *Boswellia serrata* and *Anogeissuslatifolia* with respect to other tree species in the study sites Chandlidungri

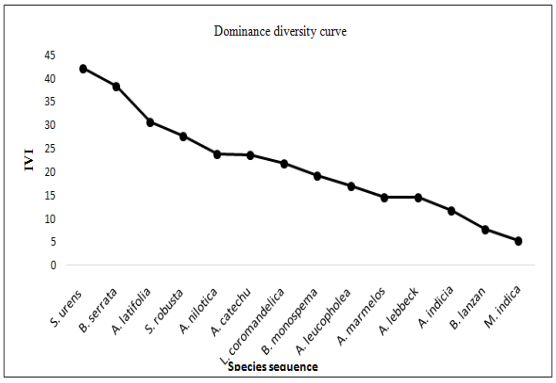

Figure-4: Dominance diversity curve for the gum yielding plants in study sites:

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RESEARCH ARTICLE

Pharmaceutical Aspects of Fucoidan; A Marine Seaweed

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ABSTRACT

An ocean is a body of water that composes much of a planet's hydrosphere that always served as a nutrient source whether from mollusks, fish or vegetarian. The animal and human populations in the world have always relied upon the sea for nutrition and sustenance. Brown seaweed has been harvested around the world for centuries. Fucoidans are the sulphated polysaccharides with a fucose backbone seen mainly in brown seaweed and account for more than 40% of the dry weight of the algal cell walls. Fucoidan from seaweeds are heterogenic mixtures andvaries their composition according to seasonal changes, the species, the climate and the extraction methods used to isolate them. This review article describes about the various applications of fucoidan such as nanoparticles, microparticles, liposomes, semi-solid formulations, cosmetics and cancer therapy.

Keywords: Brown seaweed, Fucoidan, Applications

INTRODUCTION

Marine environment and its diverse organisms are an abundant source of various compounds which have different bioactivity and biological properties. Marine polysaccharides can be explained as a large complex group containing a heterogenous macromolecules with varying biological properties. Among the different marine sources algae are a diverse group of aquatic organisms, which are the natural source of polysaccharides. These are also known as seaweeds. Based on the exhibited photosynthetic pigments such as red, brown and green, marine algae can be categorized into three groups. Sulfated polysaccharides are one of the special group stands out among the different marine polysaccharides. Fucoidan is a sulfated polysaccharide from the source of marine brown algae, marine invertebrates, sea urchins eggs and sea grasses.[1,2,3] In 1913, it was first isolated from marine brown algae by Kylin

and was named as "fucoidin". Later according to IUPAC rules, it is named as "fucoidan" but it is also called as fucan, fucosan or sulfated fucan. When the toxicological area of fucoidan studied, in-vitro and in-vivo evaluations shows that fucoidan is non toxic. Also when conducted animal model studies at a high level of ingestion, fucoidan derived from *Undaria pinnatifida* and Laminaria japonica was found to be safe.[4,5] In clinical studies, oral safety of fucoidan was determined. At a dose of 1g per day upto three months[6] or 3g per day for 12 days fucoidan shows non toxicity in human clinical studies by oral ingestion.[7,8]

FUCOIDAN FEATURES

Fucoidan is hygroscopic in nature with a yellow or pale brown colour powder. In water, it is easily soluble but not in organic solvents. Fucoidan is not usually used for the purpose of a thickening agent since its aqueous solutions are not viscous. Some of the main factors which affect the viscosity of fucoidan aqueous solutions are pH, temperature, number of sulfate groups, molecular weight, degree of branching of molecule etc.. One of the another main factor which have an impact on the viscosity of fucoidan is by algae species. When comparing the solutions of fucoidan from *Fucus vesiculosus*, *Saccharina longicruris* and *Ascophyllum nodosum*, the highest viscosity was seen from the solution obtained from *Fucus vesiculosus*[9]. When comparing with other polysaccharides gelation ability was not seen by fucoidan. Based on the electrostatic interactions, when it is mixed with an oppositely charged polymer, gels can be obtained.[10,11]

APPLICATIONS OF FUCOIDAN

Nanoparticles

The spherical particles which have a diameter range from 10 to 1000nm are categorized as nanoparticles. According to the method of placing the active substance and its composition nanoparticles are categorised as nanospheres and nanocapsules. Nanospheres are solid polymers usually in which drugs embedded in the polymer matrix. Nanocapsules are the shell which have an inner space loaded with the drug which have to be placed. In the modern pharmaceutical technology, nanoparticles are one of the important multi compartment carriers since they act as a protectant of the active moiety against enzymatic and chemical degradation and also provide a sustained or targeted release of drug. The two methods which are adopted to utilize the fucoidan for the nanoparticle preparation are ionotropic cross-linking and self assembly. As the fucoidan is negatively charged, it gets interacted with the positively charged polymer for the ionotropic cross linking.[12] In the case of self assembly method, two molecules interact with each other and form themselves into specific structures and no need of any additional elements.[13]

There are several articles published in many journals about the nanoparticle formulation with fucoidan. A short description about some of the selected articles which portrays nanoparticle formulation are included here. Fucoidan have the ability to reduce the toxicity and for increasing the stability of metallic nanoparticles. Doxorubicin-fucoidan-gold nanoparticles composite shows a significant decrease in the viability of rabbit squamous cells carcinoma [VX2]. The above mentioned nanoparticle composite and laser irradiation treatments were done in rabbits and the viability of examined cells was observed. The invivo studies conducted in rabbits which have eye tumor showed similar conclusions. The group of rabbits treated with nanoparticles and laser eradication was observed after 6 days and also there was no relapse after 14 days[14].

In nanoparticles the fucoidan-chitosan combination or its derivatives are very common which depicts the properties of both polysaccharides. The negatively charged fucoidan sulfate groups and the positively charged chitosan are complexed by electrostatic interactions. Chitosan have greater role in the antifungal and antibacterial activity. It also have the ability to overcome the epithelial barriers by mucoadhesion and allows the active substances transport. Both the fucoidan and chitosan are biocompatible and also have low acquisition costs.[15,16,17]. In 2012, Silva et al. compared the effect of fucoidan solutions with fucoidan – chitosan nanoparticles. By coacervation method, nanoparticles were obtained. It was biocompatible with human epithelial cells from colon adenocarcinoma and it also have the property to open the tight junctions between them. The results of the comparison study shows that

fucoidan nanoparticles were produced stronger anticoagulant effect and better permeability than the fucoidan solution. By simple self assembly method, fucoidan-chitosan composed nanoparticles were obtained to transfer antibiotics to the lungs. By the scavenging 1,1-diphenyl-2-picryl hydrazyl radicals and test of reactive oxygen species with lipopolysaccharides, the antioxidant activity of obtained nanoparticles were confirmed. The other properties like stability in saline phosphate buffer, no negative effect on the viability of A549 cells at a concentration from 0.37mg/ml to 3mg/ml and controlled release of gentamicin helps to declare that, the fucoidan-chitosan nanoparticle composite works as a safe pulmonary drug delivery system [18] By ionotropic crosslinking method, nanoparticle with gentamicin was prepared and introduced intratracheally. Same conclusions were obtained from this study also. The antioxidant and antibacterial properties of fucoidan, antibiotic release profile, results the limitation of potential nephrotoxic and ototoxic effects of gentamicin and also resulted in the growth inhibition of Klebsiella pneumonia.
As the fucoidan have ability to provide stability, silver nanoparticles were obtained by self assembly method using chitosan-fucoidan complex coated with silver nanoparticles which showed antibacterial activity against both the Gram negative (Escherichia coli) and Gram positive (Staphylococcus aureus) bacteria. Also the usage of nanoparticles at a concentration of 50$\mu g/ml$ gives considerable cytotoxicity to HeLa cells [19].

Pinheiro et al received nanoparticles by thelayer by layer method, in which an alternating layers of chitosan and fucoidan was introduced on polystyrene core. After removing this polystyrene element, poly-L-lysine which have antibacterial properties were filled in this nanoparticles. The adsorption of poly-L-lysine on the core and the pH dependent release of the active ingredient from these nanoparticles showed a satisfactory encapsulation efficiency and as a drug carrier[20]. In case of peptide therapy, oral administration is limited due to poor bioavailability and its degradation in digestive tract and poor adsorption Tsai et al. obtained nanoparticles with fucoidan and trimethyl chitosan as a carrier for insulin. As a result, better penetration across the intestinal epithelium and also the protection of insulin against pH changes was observed. Thus the fucoidan shows the ability to inhibit α- glucosidase activity. This nanoparticles inhibited the activity of this enzyme at a ratio of 33.2% and a concentration of 2mg/ml.[21]

Liposomes

Liposomes are the closed bilayer phospholipid system in 1965 having a size which does not exceed 100nm. Liposome researchers countless work over a decades led to the formation of important technical advances such as triggered release liposomes, remote drug loading, long circulating liposomes, ligand targeted liposomes, liposomes containingnucleic acid polymers, liposomes including a combination of drugs etc..This advanced drug delivery system led to a number of clinical trials in various areas for the treatment of cancer therapy, fungal infections, antibiotic drug delivery, anti-inflammatory drug delivery etc., Let us look forward about the application of liposomes with fucoidan. Fucoidan's antitumor effect was studied by a comparison study between low molecular weight fucoidan which was encapsulated in liposome and the fucoidan with a molecular weight 2-10kDa and 80kDa using human osteosarcoma cells. After the observation study, it was detected that the apoptosis induction by activating caspase pathway was stronger than the fucoidan. Almost similar conclusions were seen in the studies which was conducted in C3H mice that was injected previously with murine osteo sarcoma cells. In one group of mice was treated with fucoidan liposomes for 28 days orally and conducted a comparison study with mice receiving only water. The results shown that a significant decrease in the weight and volume of tumor. Fucoidan liposomes showed a better result and also it helps for the better penetration through the Caco-2 cell layer [22].

Semi-solid formulations

The three dimensional formulations which were made up of hydrophilic polymers with a suitable structure and properties are known as hydrogels. They are commonly used as drug delivery systems, wound dressings, implants etc.[23]. In the formation of hydrogels, fucoidan plays as a useful component due to its properties such as anticoagulant and anti-inflammatory activity. Sezer et al. obtained a hydrogel with the combination of fucoidan and chitosan. Providing adequate moisture and absorbing exudate are the two special features of hydrogels. The

formulations which contain fucoidan show greater possibility of water adsorption and higher swelling ratio than that of the pure chitosan gels. The hardness of the gels were greatly affected by the electrostatic interaction between polymers which was increased here by the addition of fucoidan. Also the formulation with a combination of chitosan and fucoidan showed greater cohesiveness and adhesion values. Fucoidan enhances a stronger mucoadhesive property. When conducted a comparative study between the hydrogels containing chitosan alone and the combination with the addition of 0.25% or 0.75% fucoidan showed the adhesion values of 23, 62 and 142$\mu J/cm^2$, respectively. Wound healing studies were conducted and evaluated in vivo, it seems to be very effective. Study conducted on the New Zealand rabbits with fucoidan-chitosan gel showed higher values of the length and thickness of epithelium. The synergistic effect of fucoidan and chitosan on the healing process was proved by the complex gel in this study due to the complete cure of wound which was occurred within 21 days.[24].

Purnama et al. obtained 3D scaffolds and evaluated the vascular endothelial growth factor and the influence of molecular weight of fucoidan in its activity. Test groups are categorized such as hydrogels prepared with low, medium and high molecular weight fucoidan and control group treated by hydrogels containing Sodium trimeta phosphate pullulan and DXT. Result showed that the hydrogel with mediummolecular weight fucoidan decreased significantly the release rate of vascular endothelial growth factor when compared to the control. After the subcutaneous injection of hydrogels to C57/BL6J mice, the synergistic effect of medium molecular weight fucoidan and vascular endothelial growth factor was confirmed. Lee et al obtained hydrogel for ocular application by poly(2-hydroxyethyl methacrylate), fucoidan and methacrylic acid using ethylene glycol dimethacrylate as crosslinking agent. These hydrogels were evaluated by determining the water absorption capacity, antibacterial properties and adsorption of proteins. Results showed that the water content of hydrogels and adsorption of proteins increased with methacrylic acid concentration and independent on the fucoidan concentration.[25]

Microparticles

Microparticles are a heterogenous group of bioactive small vesicles with sizes ranging from 1μm to 1000μm. They are categorized as microspheres and microcapsules. Among them microspheres are the small particles which have spherical shape. Microencapsulation is the process of enclosing a substance inside a vesicle called capsule and the small sphere with a uniform wall around it is known as the microcapsules. There is an internal phase and outer wall which called as shell were present in every microcapsules. Fucoidan have various role in the microparticle drug delivery systems and the microspheres which utilizes fucoidan advantages are refered as fucospheres.According to the literature reviews, it was found that the fucoidan based microparticles are obtained with additional copolymers or by positively charged polymers.[26]. In 2006, Sezer et al. conducted a study with negatively charged fucoidan and positively charged chitosan including bovine serum albumin. He explore the use of fucospheres in that work and noted that when the content of fucoidan in that fucospheres increased, the bovine serum albumin encapsulation capacity increased and also the zeta potential value decreased. The obtained Bovine serum albumin fucospheres showed a three-phasic release profile of bovine serum albumin.[27].

Also the fucospheres with a fucoidan-chitosan composite were used for the treatment of dermal burns in rabbits. On the 7th day, differences was noticed in the epithelial thickness values like 193,121,118 and 111μm for fucospheres, fucoidan solution, chitosan microspheres and untreated group respectively. On the 14th day, it was found that the epithelial lengths increased with time and the group treated with fucospheres showed highest values. Due to the characteristics of fucoidan such as re-epithelization, release of growth hormones and migration of fibroblasts showed a result in the group treated with fucospheres such as a fastest skin regeneration after burns. According to this study fucoidan and chitosan have a synergistic effect on the skin burn treatment.[28]. Sezer et al. conducted a comparative study between fucospheres and chitosan microspheres for distinguishing the antibiotic carriers features. It was prepared by polyion complexation and precipitation methods. The results showed that the encapsulation efficiency of fucospheres was greater than that of chitosan microparticles with the active moiety Ofloxacin. The fucospheres showed a slower release of Ofloxacin and it was consistent with the Higuchi kinetics model.[29].

Cunha et al conducted studies in the treatment of tuberculosis by fucoidan microparticles. His studies results helped for distinguish the ability of fucoidan to recognize macrophages in the alveoli. So that the effectiveness of the therapy increased and also helped for the delivery of drug only to the affected areas. By spray drying method, the microparticles containing isoniazid or rifabutin was produced and by the usage of Andersen cascade impactor, the appropriate aerodynamic properties were measured. Because of the good solubility of fucoidan and microparticles wrinkled surface, the release of both drugs occurred independently of the solubility of the free drug and medium pH within a short time (within 15 minutes). Microencapsulation results in the toxicity reduction of loaded rifabutin in human alveolar epithelium and human monocytic cells, which was proved by MTT tests [30].

Cancer therapy

Cancer is the uncontrolled growth of abnormal cells anywhere in a body which act as a leading cause of death. Several therapeutic strategies are possible to treat different types of cancer such as chemotherapy, radiation therapy, surgery and their combinations. The anticancer activity of fucoidan from brown seaweeds leads for the development of new therapeutic measures against cancer[31,32,33,34]. Lu et al. obtained nanoparticle using the negatively charged fucoidan and protamine which is acationic peptide by the electrostatic interactions. This complex was stable at a pH 7.4 which is corresponding to the blood pH. The release of more than 90% of anticancer agent occurs when the pH decreased to 4.5 which is the tumor cell pH or 1.5 which is stomach pH. This happens because the electrostatic interactions became weaker and so that the size of nanoparticle increased. Doxorubicin was used as the antitumor drug here and the side effects of this drug was prevented when smaller amount of drug release occurred. Also the concentration of drug increased in the tumor cell. So here a pH- dependent release profile obtained and this makes the intravenous injection as a best route of administration of these nanoparticles.[35].

Jang et al obtained coppersulphate nanoparticles by layer-by-layer technique. This coppersulphate nanoparticle was coated using fucoidan and polyallylamine hydrochloride in alternate manner. After the observation, it was found that this nanoparticles provide stronger anticancer effect than their usage as separate components. It provides a favourable photothermal feature and also improved the intracellular transport of fucoidan. Invitro studies by using Human cervical cancer cells and Human adenocarcinoma cells and the invivo studies in mice helped to confirm the results[36].

Cosmetics

Many of the studies were conducted based on the bioactive properties of fucoidan, which focused on the cosmetic purpose of fucoidan. Almost all of these research focused on the inhibitory effects of fucoidan which applied topically on photo-damaged and aged skin [45]. The dermal fibroblast proliferation and deposition of collagen and other matrix factors are enhanced by the action of fucoidan [37]. Low molecular weight fucoidan inhibited the matrix metalloprotease which modulate the connective tissue breakdown [38]. Fujimura obtained a gel formulation with 1% of fucoidan and conducted a clinical study for about five weeks by applying topically to human cheek skin twice daily. By the usage of a B-mode ultrasound it was found that a significant decrease in skin thickness was obtained and an improvement in the elasticity was seen which was measured by Cutometer [39].

C Michel et al described in his article that fucoidan have greater advantages as a topical and oral anti-inflammatory agent. It is used in case of allergic skin condition, sun burn cases, soothing products etc. Also he mentioned that it have greater stability than heparin. As it is a plant derived product, it have many advantages of its own [40]. N Li et al conducted a toxicological evaluation of fucoidan extracted from Laminaria Japonica in Wistar rats. In case of cosmetic ingredient or neutraceuticals, toxicological studies are important. So in his article he described that fucoidan can be considered as a dietary fiber and it is non-toxic in cell culture. No toxicological effects was observed in rats given upto 300mg/kg orally of fucoidan from Laminaria Japonica. Anticoagulant effects were observed at doses of 900 to 2500mg/kg, but other signs of toxicity was not mentioned [41].

Mizutani et al., conducted a study in cosmetics to develop a substance which have skin cosmetizing effect. He selected fucoidan as a degradation product, a sulphated monosaccharide to prevent skin aging, amelioriation of sensitive skin, antipruritic action and also as an effective ingredient for a hair care product.[42].Fernando IS et al., conducted a study to design a cost effective strategy to purify fucoidans from brown algae. He selected *Chnoospora minima* (CMF) and *Sargassum polycystum* (SPF) harvested in Sri Lanka and to evaluate their cosmetizing properties. Fourier transform infrared spectroscopy and monosaccharide composition analysis were performed and found that purified polysaccharides were rich in fucoidan. Evaluation studies were carried out to determine the antioxidant, UV-protective, anti-inflammatory, anti wrinkling and skin whitening effects. Both brown algaes showed 2,2-diphenyl-1-picrylhydrazyl and alkyl radical- scavenging activities, anti-inflammatory effects, collagenase and elastase inhibitory properties and skin whitening effects by direct inhibition of tyrosinase and intracellular melanin synthesis indicating cosmetizing effects [43].

CONCLUSION

In conclusion, fucoidan from brown seaweed seems to be a different product of the sea that can be of potential use. The long use within the Asian countries and the documented safety profile offers some assurance as to safety.This review describes some of the more recent research into the bioactivity and discusses potential therapies from fucoidan. Overall, the availability and safety of commercially available fucoidan preparations will lead to interesting adjunct and sole therapies in addition to providing new approaches.

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Table No. 1 MARKETED COSMETIC FORMULATIONS OF FUCOIDAN

1	Revitalizing anti-age facial cream	tian De products
2	Fucoidan serumist	Eau vera products
3	Fucoidan gel	Fuco well
4	Sea herb facial toner spray	Juvenee
5	King konboo shampoo	AFC

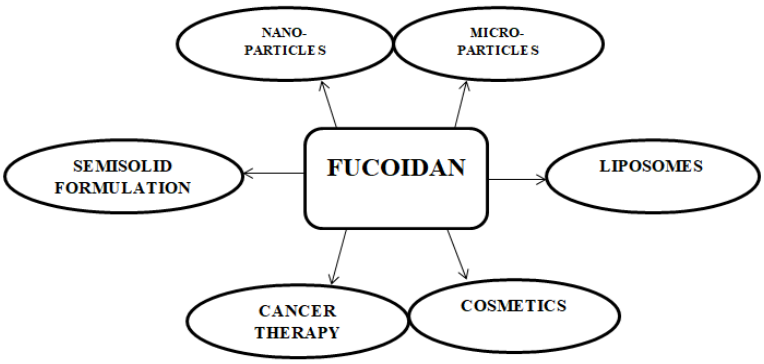

Fig 1: Applications of fucoidan

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RESEARCH ARTICLE

In vitro Protocol for Propagation Hybrid *Lilium* Cv. Fangio

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ABSTRACT

An efficient protocol was developed for the *in vitro* propagation of hybrid *Lilium* Cv. Fangio on Murashige and Skoog basal medium supplemented with plant bioregulators in the Biotechnology-cum-Tissue Culture Centre, OUAT, Bhubaneswar. The pre established calli mass derived from bulb scale explants were cultured in MS media supplemented with growth regulators in combination of BAP (0.5 mg/l) and (1.0 mg/l) with 2,4-D (0.5,1.0,1.5,2.0,2.5 mg/l) and 2,4-D (0.5,1.0,1.5,2.0,2.5 mg/l) alone with 8% (w/v) agar, 30% (w/v) sucrose with three replications each. As the results revealed after 120 days of culture among the treatments, basal media supplemented with BAP 1mg/l + 2,4-D 0.5 mg/l gave early swollen mass initiation (17.66), early bulb initiation (21.66), early shoot mass formation (23.33), earliest shoot proliferation (40.66). MS + BAP 1.0 mg/l + 2,4-D 2.0 mg/l recorded significantly maximum value for the number of leaves (5.66), height of shoot mass (5.66), followed by 150 days of culture MS + BAP 1mg/l + 2,4-D 2.0 mg/l gave highest number of shoots per shoot mass (7.66), the maximum number of bulbs per shoot mass (5.56), maximum height of the plantlet (7.98), and lastly the maximum size of the bulb (0.8cm×0.66cm) producing deep green colour compact plantlets was the best combination. Thus above recorded combinations opened many prospects for developing an indirect means of *in vitro* regeneration of hybrid *Lilium* Cv. Fangio thereby strengthening the way in which biotechnological intervention could be used for the fulfillment of the national and international demands of this cut flower.

Keywords: Bulb, callus, culture, Fangio, *in vitro*, *Lilium.*

INTRODUCTION

There are many techniques available for the conservation of plant genetic resources of ornamental species. These include micropropagation, seed germination, regeneration from callus, embryo rescue, micro-grafting, and cryopreservation [1].Through *in vitro* propagation of bulb plants an alternative way has been found to the conventional methods of vegetative propagation because of its various advantages, such as increasing the multiplication level in less time and enables material free from viruses and other diseases were obtained [2]. *Lilium* has been known as an amenable genus, which adapts to the tissue culture techniques. Plant regeneration via *in vitro* techniques can be achieved from the explants [3,4], *Lilium* species, for centuries, have been one of the horticultural most important genera for cut flower, pot plant production and are taken as the king of ornamental bulb plants. The genius *Lilium* comprises about 85 species, and as it is derived from interspecific hybridization, it acquires a great importance in the commercial market being a monocot bulbous crop. *Lilium* is also gaining popularity due to its attractive large flowers, capacity to rehydrate after long transportation and emerging as the largest income contributor [2]. 80 species of *Lilium* are found in the temperate and subtropical zones of the northern hemisphere [5]. A good number of researchers [6] did carry out studies on interspecific hybridization, for example, *L.Longiflorum* x Asiatic hybrid and have had great success in the early 1990's introducing a new class of lily called as LA (*Longiflorum x Asiatic*) hybrids to the market. Fangio belonging to LA (*Longiflorum x Asiatic*) hybrids variety were bred for the numerous desirable characteristics such as vigorous growth of bulb, healthier leaves, stems, larger flowers and elegant fragrance.

Flower bulbs have been appreciated and cultivated for thousands of years and long before they were widely grown commercially or extensively researched. Previously suggested that explants from flower organs to bulb scales of *Lilium* could be easily manipulated and regenerated using tissue culture techniques **[7]**. *Lilium* propagation is usually done by vegetative means which produces 3-4 bulbs per bulb scale depending on size and variety. The multiplication efficacy by bulb is low and the plantlets are more susceptible to diseases. Thus this study is of significance as it by passes bulb production. Through tissue culture, there is not only continuous supply of bulblets but true-to-type and disease free plants can be obtained. *In vitro* techniques have become increasingly important in the production of good quality and quantity of crop. Therefore, the objective of the present research was to establish a protocol for standardization of plant bio regulators through *in vitro* techniques in *Lilium* hybrid Cv.Fangio.

MATERIALS AND METHODS

The present investigation was carried out during the year 2013-14 at Biotechnology-cum- Tissue Culture Centre, OUAT, Bhubaneswar, Odisha.

Source of Explants

For this study, healthy and disease free explants were collected from the hybrid *Lilium* Cv. Fangio from Agro-shade net house, in the of Biotechnology-cum-Commercial Tissue Culture Center, OUAT, Bhubaneswar, Odisha (Fig.1).

Stock solution, Media preparation and Sterilization

The chemicals used for the present study were analytical reagents of excel R grade of Titan Biotech Ltd., Ranbaxy Laboratory Ltd., Merck (India),Qualigen Fine Chemicals, and Himedia Laboratories Ltd. (India). Auxins, Cytokinins, myo-inositol and Fe-EDTA that were supplied by Sigma (USA) and Agar from Ranbaxy Laboratory Limited. MS Medium (Murashige and Skoog, 1962)**[8]** was used throughout the investigation, required quantities of macronutrients, micronutrients, Fe-EDTA, vitamins and plant bioregulators were taken from the stock solution and required quantity of sucrose dissolved in distilled water was added fresh to the medium. The pH of the solution was adjusted to 5.7+ 0.1 using 0.1N NaOH or 0.1N HCl. Then volume was made up to 1 Lt. with distilled water. Agar (0.6% w/v) was

added to the medium boiled and poured into the culture tube followed by plugging. Culture tube containing culture medium was autoclaved for the 20 minutes at 121^{0}C and 15 Psi pressure. The autoclaved medium was kept in laminar air flow bench for cooling. All the glassware was dipped in detergent solution for overnight and washed under running tap water. They were rinsed with distilled water and then dried in oven for 2hrs at 150^{0}C. Forceps, petridish and scalpel were thoroughly cleaned with iso-propanol, rapped with paper and kept in a clean sterilized autoclave at 15 psi and 121^{0}C for 20 minutes. The working chamber of laminar air flow cabinet was wiped clean with iso-propanol. Filtered air (80-100 cft/min) was blown for 5 minutes, to ensure that particles do not settle in the working area. The sterilized materials to be used (except living tissue) were kept in the laminar chamber being exposed to UV light for 30 minutes.

Inoculation

For callus formation study, bulb scale explants were treated with 0.1 % $HgCl_2$ for 5 min, followed by culturing in MS media supplemented with plant bioregulators for two culture periods consisting of 60 days giving sufficient amount of profuse calli mass serving as explant for in vitro culture of plantlets. After that each section of the bulb scale (7 × 7 mm), with the dorsal side in contact with the medium, was placed in a culture tube with MS (Murashige and Skoog, 1962)[8] medium containing 2,4-D (0.5, 1.0, 1.5,2.0 and 2.5 mg/l) alone and BAP (0.5and 1.0 mg/l) in combination with 2,4-D (0.5, 1.0, 1.5,2.0 and 2.5 mg/l)and a control. Observation on days to swollen mass initiation, days to bulb initiation, days to shoot mass initiation, days to shoot proliferation recorded from 120 Days after inoculation (DAI) and recorded at 150 DAI.

Establishment of culture

After inoculation, the culture was kept at 25±2 ^{0}C in an air-conditioned room with 16 hours light period (3000-3200 lux) supplied by fluorescent tubes and 80% relative humidity [9].

Statistical analysis

The raw data obtained during the experimental observations were subjected to statistical analysis as per method by Gomez and Gomez, (1984)[10]. The significance and non- significance of the treatment effect were judged with the help of 'F' variance ratio test. Calculated 'F' value was compared with the table value of 'F' at 5% level of significance. The data were transferred from where ever required before the suitability of Analysis of Variance (ANOVA) analyzed in statistical package SAS version 7.0.

RESULTS AND DISCUSSION

The results presented in Table.1, Fig.2, it was revealed that for *Lilium* hybrid cultivar Fangio in T_{12} (MS + BAP 1.0 mg/l + 2,4-D 0.5mg/l) gave the earliest shoot mass initiation with 17.66 days, also with a significant early bulb initiation with 21.66 days. Both the data recorded were seen significantly at par with T_{13} (MS + BAP 1.0mg/l + 2,4-D 1.0 mg/l). The plant growth regulators stimulated various types of callus in the culture [11]. Kaur *et al.* (2006)[12] also obtained bulblets/ explants on MS medium supplemented with BAP. It was observed that the cytokinin concentration increases the number of bulblets which also in alignment with Barpanda *et al*. (2018)[13, 14]. The treatment T_{12} showed similar results for shoot mass initiation as 23.33 days as well as earlier shoot proliferation from shoot mass being 40.66 days significantly being at par with T_{13} and T_{14}. Whereas T_{15} (MS + BAP 1.0 mg/l + 2,4-D 2.0 mg/l) gave significantly maximum value for number of leaves (5.66), height of shoot mass(5.66), being significantly at par with T_{16}. As from the data represented in Table.2, it was clearly recorded, T_{15} (MS + BAP 1.0 mg/l + 2,4-D 2.0 mg/l) gave a significantly high number of shoots per shoot mass (7.66), maximum number of bulbs per shoot mass (5.56), maximum height of the plantlet (7.98), and lastly the maximum size of the bulb (0.8x0.66) producing deep green color compact plantlets. These data were seen at par with T_{16} as well respectively. Shoot differentiation was also high in MS medium supplemented with bioregulators[15]. The results are also in concurrence with the observation made by Maesato *et al*.(1991)[16] in *Lilium japonicum* [17,18].

CONCLUSION

The study is significant as it enhances the mass production of Lilum Cv. Fangio plantlet production, further it can successfully demonstrate in simple, rapid methods of producing healthy plantlets under the aseptic condition. Hence this investigation opens the way for uses of biotechnological techniques have an increase in the production of good quality and quantity of this crop.

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Table 1. Effect of plant bio-regulators on days to swollen mass emergence, bulb formation, shoot and plantlet formation from developed callus in Hybrid *Lilium* hybrid Cv. Fangio

Basal Medium- MS **Culture period - 120 days**

Characters / Treatments	BAP	2,4-D	Day to swollen mass initiation	Days to bulb initiation	Days to shoot mass initiation	Days to shoot proliferation	Number of shoots/ mass	Height of shoot mass	Number of leafs per shoot mass
T_1	-	-	52.00	55.66	57.66	69.66	2.10	1.00	1.00
T_2	-	0.5	53.33	56.00	58.00	70.33	2.18	1.33	1.00
T_3	-	1	54.00	56.33	58.66	71.00	2.40	1.66	1.66
T_4	-	1.5	54.33	56.66	58.66	71.66	2.62	2.33	1.66
T_5	-	2	55.00	58.00	60.00	73.33	2.81	3.00	2.66
T_6	-	2.5	54.33	58.00	60.00	73.33	2.76	2.66	2.33
T_7	0.5	0.5	21.33	25.66	27.66	42.33	4.06	2.33	2.00
T_8	0.5	1	22.66	26.33	28.33	42.33	4.20	2.66	2.33
T_9	0.5	1.5	24.00	28.00	29.33	44.66	4.30	3.66	2.66
T_{10}	0.5	2	26.66	28.66	30.00	46.00	5.02	4.33	3.66
T_{11}	0.5	2.5	25.66	28.33	29.66	45.33	4.8	3.66	3.33
T_{12}	1	0.5	**17.66**	**21.66**	**23.33**	**40.66**	4.2	2.00	2.66
T_{13}	1	1	17.66	22.66	24.66	43.66	4.87	2.66	2.66
T_{14}	1	1.5	20.33	25.33	27.33	44.66	4.90	3.66	3.66
T_{15}	1	2	24.33	28.33	30.00	46.66	**5.08**	**5.66**	**5.66**
T_{16}	1	2.5	23.00	27.33	29.33	46.00	5.00	5.33	5.33
SE(m)±			0.28	0.29	0.31	0.25	0.04	0.30	0.30
CD 5%			0.78	0.82	0.88	0.71	0.12	0.85	0.85

Table 2.Effect of Plant bio-regulators on bulb formation, shoot and plantlet formation from developed callus at 150 days in Hybrid *Lilium* hybrid Cv. Fangio

Basal Medium- MS **Culture period - 150 days**

Characters / Treatments	BAP	2,4-D	Number of shoots per shoot mass	Number of bulbs per shoot mass	Height of the plantlet	Size of the bulb
T_1	-	-	2.00	1.33	4.10	0.1×0.49
T_2	-	0.5	2.33	1.66	4.28	0.1×0.54
T_3	-	1.0	3.33	2.00	4.46	0.1×0.66
T_4	-	1.5	3.00	2.33	4.69	0.1×0.84
T_5	-	2.0	3.66	2.66	4.83	0.2×0.51
T_6	-	2.5	3.33	2.33	4.80	0.2×0.44
T_7	0.5	0.5	3.33	2.33	6.06	0.1×0.70
T_8	0.5	1.0	3.66	3.00	6.28	0.2×0.60
T_9	0.5	1.5	4.33	3.66	6.33	0.2×0.75
T_{10}	0.5	2.0	5.66	4.66	6.87	0.4×0.35
T_{11}	0.5	2.5	4.66	4.00	6.69	0.4×0.38
T_{12}	1.0	0.5	3.33	3.33	6.27	0.2×0.25
T_{13}	1.0	1.0	4.00	3.66	6.54	0.2×0.52
T_{14}	1.0	1.5	4.66	4.33	6.74	0.3×0.69
T_{15}	1.0	2.0	**7.66**	**5.66**	**7.98**	**0.8×0.66**
T_{16}	1.0	2.5	6.33	4.66	7.59	0.6×0.57
SE(m)±			0.30	0.30	0.08	
CD 5%			0.12	0.1	1.75	

Fig.1 View of Hybrid Lilium hybrid Cv. Fangio

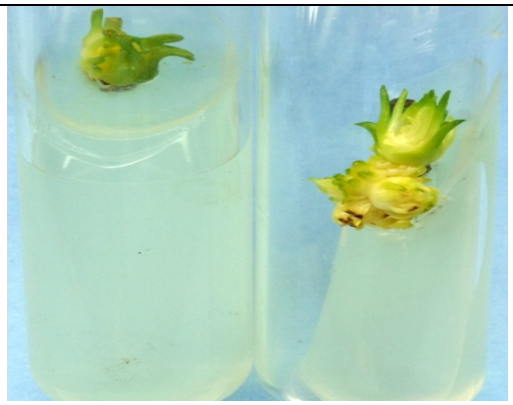

Fig. 2 Shoot mass initiation from bulb-scale explant

Fig. 3 Bulb mass initiation

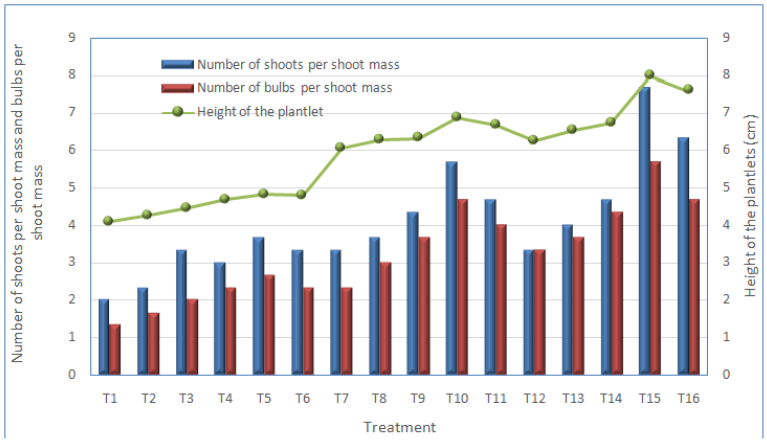

Fig .4 Impact of plant bioregulators on number of shoots per mass, number of bulbs per shoot mass and height of plantlet after 150 days of subculture for hybrid *Lilium* cv. Fangio.

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RESEARCH ARTICLE

Free Floating Hydrophytes and Their Utility: An Appraisal

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ABSTRACT

Aquatic plants are very remarkable forms of plant life and are essential component of the wetland ecosystem. A variety of products and significant beneficial services are offered by these groups of organisms. Hydrophytes are classified based on their living pattern in certain environment with food, water, shelter and space to survive and their morphological form, size and developmental properties in wet land. The most common floating aquatic plants in wetlands are *Azolla*, *Eichhornia*, *Pistia*, *Salvinia*, *Lemna*, *Spirodela* and *Wolffia*. Free-floating plants, the group of primary producers of an aquatic ecosystem, sometimes create problems under certain conditions. On the other hand, these free-floating plants play an important role in wetlands by providing shelter, oxygen and carbon dioxide to other aquatic organism. Human beings have been using these plants as food, medicine, biofertilizer and in biogas or biofuel production and aquaculture from many years (since ancient times). These plants can also soak up pollutants from contaminated water. Therefore, understanding the responses of floating plants to its biotic and abiotic environmental conditions will throw some light on the management and beneficial applications of these plants. This review paper examined the various useful aspects of the native free floating aquatic plants and their future prospects.

Keywords: Aquatic macrophytes, Free-floating, Utility

INTRODUCTION

A major fraction of the total biomass in an aquatic environment comprises with the photosynthetic organisms, both the phytoplankton and macrophytes [1]. The plants which grow actively in aquatic environment are called as hydrophytes [2]. According to the plant's position with respect to the water surface there are four main life forms of hydrophytes, such as;

1. Free -floating hydrophytes
2. Rooted hydrophytes with floating leaves
3. Rooted submerged hydrophytes
4. Rooted emergent hydrophytes

Aquatic plants are essential in promoting the diversity and function of aquatic ecosystem and also provide many ecological benefits [3]. Economical and socio-cultural worth of floral diversity is well known throughout the world as it provides food and medicine to us [4]. Nahlik and Mitsch in 2006 reported that the floating aquatic macrophytes are the most dominating plants in tropical wetlands [5]. The floating aquatic plants are also helpful to other aquatic life by providing them shelter, nutrition, protection and habitat for their reproduction. They purify the water bodies by absorbing pollutants from it and they are also helpful in production of oxygen [6]. The various usages of some widespread free floating hydrophytes are discussed below.

METHODOLOGY FOLLOWED

A detailed and comprehensive literature review was made by searching various websites as well as relevant research articles published besides the study materials available at different educational and research institutes including Centurion University of Technology and Management, Odisha, India. Various proceedings of the National and International seminars, workshops and symposia on hydrophytes or aquatic plants were referred with regards to their different utilities. The search keywords such as hydrophytes, floating macrophytes, phytoremediation, biogas production, biofertilizers, organic manures etc. were used for finding out related research articles for the preparation of updated review article.

RESULTS AND DISCUSSION

The results of the review study revealed the reports on the salient features, occurrence and distribution of some common free-floating hydrophytes and evaluation of their various applications including as feed and fodder, medicine, biofertilizer, organic manure, waste disposer and bio-resources for biogas as well as biofuel production. The present review article enumerates the updated information and documentation of some familiar free-floating macrophytes used for various purposes. The results of the present exhaustive literature survey indicated that the data on 10 plant species belonging to 8 genera and 5 families utilized as human food, animal feed, medicine, biofertilizer, water purifier as well as the production of biogas and biofuel. It was also found that 7 of these plant species belonged to the angiospermic group while 3 under Pteridophytes. Analysis of the data indicated that the whole plant or part (s) of the plant have been used in different ways as mentioned below.

Enumeration

Azolla pinnata R.Br.

Azolla is a genus of free floating aquatic fern belongs to the family Azollaceae, commonly found in tropical and warm temperate regions (Fig. 1). The branched stem of this plant floats horizontally on the water surface. The inner surface of each leaf cavity of this plant is lined with an envelope [7] and multicellular transfer hairs [8].

Eichhornia crassipes (Mart.) Solms.

Eichhornia crassipes (Mart.) Solms., a hydrophyte plant belongs to family Pontederiaceae is commonly known as water hyacinth (Fig. 2). It is the most productive invasive plant on earth. It has been originated from the American tropics and now found in all tropical countries [9]. According to Jafari (2010) warm water bodies rich in macronutrients is the best condition for growth of water hyacinth and can tolerate a wide range of environmental condition and capable to reproduce in shallow water condition[10].

***Pistia stratiotes* L.**

Pistia stratiotes L. commonly called as water lettuce belongs to the family Araceae (Fig. 3). Water lettuce is a free floating aquatic plant that grows in ponds, streams, lakes and is also called as water cabbage or shellflower. The leaves are light green with parallel venation, wavy margins and have short hairs. Whitish hairs are present on the lower surface of the leaves [11].

***Salvinia molesta* D. Mitch. and *Salvinia cucullata* Roxb. ex Bory**

South-eastern Brazil is the native place for *Salvinia* (family-Salviniaceae) where it grows between latitude 24° to 32° (Fig. 4 & Fig. 5). The giant *Salvinia* is found in tropical and sub-tropical regions of more than 20 countries and in some places used as an aquarium or water garden species. The floating leaves possess distinct midribs, hydrophobic hairs on the upper surface and castaneous hairs on the lower surface. The rhizomes bear leaves in whorls of three and their growth is through horizontal branching of rhizomes [12].

Duckweeds (*Lemna*, *Spirodela*, *Wolffia*)

A study performed by Landolt in the year 1986 indicates that duckweeds are the group of the free floating aquatic plants, capable of multiplying rapidly through vegetative budding of new fronds and their rate of biomass accumulation is more than other aquatic plants [13]. These are the smallest and simplest flowering plant. The growing period of duckweeds is longer than other aquatic plants. Duckweeds can grow through all seasons in areas with warmer climatic condition.

***Lemna minor* L.**

Lemna is a C-3 type monocot plant, commonly called as duckweed and are widely distributed all over the world and are the key component of aquatic food web (Fig. 6). This free floating aquatic plant belongs to the family Lemnaceae and has a faster growth rate at the water temperature between 6 ^{0}C to 30 ^{0}C also their growth rate depends upon varieties of stresses such as toxins, extreme pH and temperature [14,15].

***Spirodela polyrhiza* (L.) Schleid.**

Spirodela are small, free-floating aquatic plants (Fig. 7) belongs to family Lemnaceae consist of connected fronds, leaf-like structure arising from the fused stem and leaves have several roots on the lower surface of each frond [16].

***Wolffia* (L.) Horkel ex Wimm.**

Wolffia, free floating rootless duckweed is a member of Lemnaceae family (Fig. 8). These plants possess fronds (or leaves) only. Species of *Wolffia* are found in stagnant fresh water bodies from tropical to the temperate region and hence they are cosmopolitan in distribution [17].

Floating hydrophytes and their Applications

The free floating hydrophytes like *Azolla*, *Eichhornia*, *Pistia*, *Salvinia*, *Lemna*, *Spirodela* and *Wolffia* have many useful applications. These plants can be utilized as human food, animal feed, medicine, and biofertilizer and water purifier. They may also be used for the production of biogas and biofuel.

As Food and Fodder

A number of hydrophytes are found rich in minerals and vitamins. Including from free floating to fixed floating and from submerged to amphibious all having nutritional value though these properties of hydrophytes have not been adequately explored. The wild edible hydrophytes such as *Echinochloa stagnina*, *Eichhornia crassipes* and *Ceratophyllum demersum* were observed to contain substantial amount of nutrients like Na, Ca, Mg, N, K, C and carbohydrate as well as proteins [18]. *Hydrilla verticillata*, a submerged aquatic plant is also appreciated by several researchers for its amazing nutritional properties [19]. Like some other fixed floating hydrophytes, *Nelumbo nucifera* has also been used as food since long.

Van Hove in 1989 experimented on *Azolla*, used as a supplementary food for a variety of animals like pigs (in China), rabbits, chickens, ducks (in Senegal) and fish (in China) [20]. In a study on broilers, Ali and Leeson (1995) found that the growth and body weight values resulted by the use of *Azolla* feed is similar to the use of maize-soybean meal [21]. Das *et al.,* (1994) reported that after biogas production from *Azolla pinnata* R.Br., the remaining digested slurry can be used as a fish pond fertilizer, which can increase the phytoplankton population at a higher rate than the use of either digested or raw cow dung [22]. They also found that by mixing the conventional fish feed and digested *Azolla* slurry in the ratio of 4:1, the growth rate of fish increases significantly. A study was conducted on lactating cows by Nik-Khan and Motaghi-Talab in 1992 and they found that by the use of *Azolla* as a feed ingredient (constituting up to 35%), the milk yields and fat percentages can be maintained at the same level as compared with conventional feeds but production levels, however, were not increased [23].

Abdelhamid and Gabr (1991) have reported that both the species of water hyacinth (*Echinochloa stagnina*, *Eichhornia crassipes*) contains 20% crude protein of their dry weight and can be utilized as a good source of animal diet [24]. According to Igbinosun and Talabi (1982) water hyacinth with specific concentration in combination with other feed has been proved to be a good quality protein source for animal diet [25]. Similarly *Pistia* has been using as feed for swine and buffalos in some countries from many years [26]. Gena *et al*., in 2014 conducted a study on the growth rate of broilers by using *Salvinia molesta* D. Mitch. as feed for them and observed that 6% of *S. molesta* in the Lohman broiler diet had a favorable and similar effect on chickens with respect to the control diet [27].

As a natural protein source duckweed has better array of essential aminoacids than other plant proteins and more closely resembles to animal protein [14]. Studies on duckweed as fish food was conducted by Robinette *et al*. (1980) and they found that duckweed had positively affected on weight gain of certain fishes, crab and tilapia [28]. Mbagwu *et al*., (1990) reported that duck weed contains about 35-45% crude protein (CP) with good amino acid and mineral profile; hence it is a good food source for fish [29]. Oron (1994) has shown that the ammonia present in domestic wastewater can be converted into valuable protein rich biomass by duckweeds, which can be used as animal food [30]. According to Lenge *et al*., (1995) duckweed growing in nutrient rich water are valuable dietary supplement for fish as it contains high concentration of trace minerals, phosphorus (P), potassium (K) and pigments like carotene [31]. The presence of little amount of fiber and indigestible material as compared most other plants has proven as good supplementary food for monogastric animals [32]. Baidya and Patel (2017) reported on high protein content and favorable amino acid pattern of duck weed can be used as a better protein source for fishes [33]. Rusoff *et al*., (1980) reported that the villagers in Burma and Northern Thailand have been using the small duckweed, *Wolffia arrhiza* (L.) Horkel ex Wimm. as a part of their diet from many years [34]. Appenroth and Augsten in 1996 described about the uses of *Wolffia*, a duckweed, as animal and human food [35]. Also countries like China and Taiwan has been growing *Wolffia arrhiza* (L.) Horkel ex Wimm. as fresh carp food [36]. It is reported that recombinant proteins can be produced from *Wolffia* by cultivating them in bioreactors [37].

In Phytoremediation of waste water

Water pollution of urban and industrial areas has become a growing concern throughout the globe. It is reported that 70% of the surface water is polluted by sewage and industrial effluents [38]. It is observed that aquatic weeds can absorb and incorporate the inorganic and some organic compounds as well as heavy metals from the polluted water into their own structure thereby behaving like a nutrient sink [39]. Therefore, a biological system involving some selected hydrophytes (phytoremediation) should be used as a supplement to the conventional physico-chemical wastewater treatment system as a 'final polish' or can be used as an alternative. A number of heavy metals such as Chromium, zinc, iron, manganese and copper are required in very small amount for both human body and plant growth. But higher accumulation of these metals is said to cause poisoning that lead to diseases. Eradication of these heavy metals from the contaminated soil and water can be possible by using hydrophytes. It was found that free floating hydrophytes take major role in remediating the polluted water without harming themselves. A number of common floating hydrophytes such as *Azolla pinnata*, *Azolla microphylla*, *Lemna monor*, *Pistia stratiotes, Spirodela polyrrhiza*, *Salvinia cucullata* and *Salvinia molesta* are reported to have hyperacumulating ability to remediate waste

waters including sewage, industrial effluents and mine's waste water contaminated with heavy metals such as chromium, nickel, lead etc. along with other hazardous pollutants [40, 41, 42, 43, 44, 45, 46, 47, 48]. Heavy metals like Iron and Copper, if present at low concentration in water bodies can be effectively removed by *Azolla pinnata* R.Br. and *Lemna minor* L. [49]. Highly polluted water generated from factories can be purified by a mixed culture of *Lemna* and *Azolla* (ratio of 2:1), up to a level that the water could be used for agricultural purposes [50].

Reddy and Tucker (1983) noticed that *Eichhornia crassipes* (Mart.) Solms. is one of the commonly used vascular aquatic plants in nutrient recovery from sewage water and from agricultural waste water [51]. Water hyacinth has high nutrient accumulation capacity which has been proved during laboratory analysis by Soerjani (1984) [52]. According to a report from US EPA (1988) in case of single pond wastewater treatment, aquatic plants such as *Eichhornia crassipes* (Mart.) Solms. and duckweed (*Lemna* species) are more effective than others [53]. Ogunlade (1992) has shown that water hyacinth works as an effective agent in accumulating heavy metals like Cadmium, Mercury and Nickel as well as other chemical substances such as Nitrates, Phosphates, Ammonia, Silicate, Chlorine and Sulphur [54]. Due to the high nutrient absorbing ability these species can be used for the secondary and tertiary treatment of waste water [55]. It can also be used for removal of organic matter, fecal coli forms and heavy metal from polluted water sources [56, 57].

Lu *et al*., in the year 2008 conducted an experiment on storm water treatment by hydrophytes and found water lettuces are more capable in improving water quality by reducing suspended solid particles, turbidity and removing Nitrogen & Phosphorus from storm water [58]. Study results of Thilakar *et al*. (2012) reveals that *Pistia stratiotes* L. is more efficient in removal of copper at higher concentration in the solution whereas *Salvinia natans* (L.) All. is effective in low concentration [59]. Moore and Ramamoorthy in 1984 have shown that *Salvinia molesta* D. Mitch., a free floating hydrophyte can be used for the treatment of black water effluents [60]. Results of a study conducted by Outridge and Hutchinson (1990) indicated that *Salvinia auriculata* Aubl. as a bio-indicator of presence and contamination level of Cadmium (Cd) in aquatic ecosystem [61]. Thilakar *et al*., (2012) have reported that *Salvinia natans* (L.) All. is more efficient in Chromium accumulation than *Pistia stratiotes* L. [59]. It is also found that heavy metals like Chromium (Cr), Cadmium (Cd), Lead (Pb) and Copper (Cu) can be removed from industrial effluents by *Salvinia molesta* D. Mitch. [62].

Oron (1994) has reported that duckweed has been used for more than a decade in tertiary treatment of industrial and municipal waste water [30]. In the year 1988 Oron *et al*., found that duckweed are useful in nutrient recycle from domestic waste water and in case of ammonium removal, duckweeds are more preferable than other macrophytes [63]. A study was conducted by Korner and Vermaat (1998) on duckweed and they found that the use of duckweed is a cost effective and easy method to handle in waste water treatment because of their high growth rate and high nutrient removal capacity [64]. According to Landolt (1986) the concentration of Nitrogen and Phosphorus in duckweeds increases with the increase in the concentration of the medium [13]. Dasgupta *et al*. (2008) reported that duckweeds have been utilized to treat agricultural and municipal waste water as these can accumulate and metabolize the pollutants [65]. Study on Lemnaceae by Culley *et al*., (1981) indicates that various types of foreign chemicals can be accumulated by the plants of this family effectively [66]. Zayed in 1998 studied on *Lemna minor* and found that it has great potential for the removal of Cadmium, Copper and Selenium from contaminated water [67].

According to Pernial *et al*. (1998) *Lemna minor* L. in monoculture method is capable to accumulate Ammonia and Phosphorus from storm water in significant amount [68]. From a study Leblebici and Aksoy in 2011 have concluded that *L. minor* is more effective than *Spirodela polyrhiza* in case of lead accumulation from polluted water bodies [69]. Kara (2004) studied on *L. minor* L. and found that it has ability to accumulate Copper and Cadmium from contaminated water [70]. Ali *et al.* (2015) reported that duckweeds, the small floating aquatic plant like *Lemna* is better in heavy metal removal from waste water [71]; and it also accumulates Cadmium (Cd) in considerable amount [72]. According to Arias *et al*. (2016) from their previous studies on waste water treatment by *Lemna* plant in pig farm have shown a decrease in the level of total suspended solids and biological oxygen demand (BOD) in the waste

water [73]. Toyama *et al*. (2006) reported that biodegradation of single aromatic compounds present in waste water can be done by *Spirodela polyrhiza* (L.) Schleid., a giant duckweed [74]. Lizieri *et al*., (2011) conducted a study and concluded that in case of Manganese (Mn) accumulation from wastewater *Spirodela polyrhiza* (L.) Schleid. has great potential followed by *Salvinia minima* Baker and *Azolla caroliniana* Willd. [75]. Aravind *et al*. (2015) conducted a study and found that Cadmium (Cd) accumulation rate of *S. polyrhiza* and *L. minor* can be enhanced by the use of a chelating agent, EDTA in the polluted water [76]. According to Hillman and Culley (1978) *Wolffia* is an excellent plant for phytoremediation of heavy metals for its high growth rate and potential to accumulate large amount of pollutants from waste water [14]. According to Boonyapookana *et al*., (2002) *Wolffia globosa* (Roxb.) Hartog & Plas., the smallest plant in duckweeds has a potential to accumulate Cadmium (Cd) from polluted water [77]. Arsenic removal from water by the duckweed *W. globosa* was performed by Zhang *et al*. (2009) and they found that this plant can tolerate up to 400 mg arsenic/Kg1 dry weight and can accumulate >1000 mg of arsenic/ Kg1 dry weight [78].

As Medicine

A specific character of the hydrophytes is adaptation which helps the organism to survive in a particular changing habitat. Such adaptation quality of these plants is beneficial in various habits specifically which can not disturb much in the growth pattern of plant and also the metabolic pathway of that specific plant. The acid pathway which is the major synthetic mechanism for secondary metabolites will not be disturbed by any changes in environment. These hydrophytes can be used as waste to wealth by exploring their medicinal properties. A large number of works have done in the field of antimicrobial activity using these plants. Several studies have been carried out in the field of pharmaceutical sector keeping in view on the herbal medicine. In case of hydrophytes it is less explored. A small aquatic fern namely *Azolla* has been used effectively as cough medicine [7]. The methanolic extract of the *Azolla pinnata* was proved to be containing high amount of secondary metabolites as compared to *Azolla rubra* and can be used to treat dental caries as evidenced by wider inhibition zone in *Streptococcus mutans* colonies and low MIC value. Duncan test concluded that *Azolla* extract can be used to inhibit the growth of *Salmonella typhii* bacteria so that it can be developed into traditional medicine.

Sanitha (2005) reported that anti-inflammatory and anti-ulcerogenic effects of *Eichhornia crassipes* in rats have been found from its methanolic extracts [79]. Earlier study indicates that the extracts of this plant shows antimicrobial (bacterial and fungal), anti-algal (green microalgae and cyanobacteria), antioxidant activities as well as wound healing and larvicidal activities in test animals [80, 81]. Further it was found that the activities of antitumour in mice can be possible by using this aquatic plant [82]. According to Zennie and McClure (1977) *Pistia stratiotes* L., a free floating hydrophyte contains large amount of two di-C-glycosylflavones of the vicenin and lucenin and lesser amount of the anthocyanin cyaniding-3-glucoside, a luteolin-7-glycoside, and traces of the mono-C-glycosyl flavones, vitexin and orientin which are responsible for wound healing activity [83]. Mukhtar and Tukur (2000) reported about the medicinal properties of *P. stratiotes* to treat nervous disorders, intestinal bacterial infections, fever, stomach disorder and in throat and mouth inflammation [84]. According to Kirtikar and Basu (2001) *Pistia stratiotes* L. is one of the important plants for Medicare purpose as its leaf extract boiled in coconut oil is helpful in chronic dermatitis [85]. Other uses such as its ash when applied to scalp helps in curing ringworm, its leaf extract helps in eczema, leprosy, ulcers, piles, and syphilis. Mukhtar and Huda (2003) reported that the ethanol and hot water fractions of the plant *P. stratiotes* exerts antimicrobial activities on a few pathogenic bacteria and the chloroform fraction of the same plant have both antifungal and antibacterial activities on some pathogens [86]. Jha *et al*., (2012) have studied on the wound healing activity of *P. stratiotes* L. on mice and found better result in a cost effective way, hence it will be a friendly drug for poor people [87].

Moghal *et al*. in 2013 conducted a study on *Salvinia minima* Baker and *Dactyloctenium austral* Steud. and found that the aqueous extracts of both plants by hot plate method had significant analgesic effect in the experimental animals as compared to the reference drug [88]. Shankar and Mishra (2012) have reported that *Lemna minor* L. has many medicinal properties including antiscorbutic, astringent, depurative, diuretic, ophthalmic, sedative, febrifuge and

they also found that the leaves of *Spirodela polyrhiza* (L.) Schleid. have antipyretic, cardiotonic, carminative and diaphoretic activities and the paste of leaves can be applied externally on measles [89].

As Biofertilizer/ Organic Manure

The tendency to look forward only the high yielding of food crop by using chemical fertilizer and pesticide for ever increasing population has no doubt saved from hunger. But at the same time the ignorance to the hazardous effect of these chemicals make environment in a critical condition. Use of green technology for agro-economical development is the best solution for these problems. Biofertilizer or organic manures is a cost effective, eco-friendly and easy way to use by the poor and marginal farmers in their field to increase crop production. The nutrient content of aquatic weed has proven to be used as the source of nutrient as well as organic matter in crop field which can help increasing crop productivity without harming health of the soil.

Singh et al., (1981) have shown that the application of *Azolla* increases the total Nitrogen, organic Carbon and Phosphorus level in soil [90]. Wagner (1997) reported that *Azolla-Anabaena* biofertilizer can be beneficial in increasing productivity of a variety of crops like rice, taro, wheat and many others [7]. It is also reported that *Azolla* is a water fern, symbiotically associated with a nitrogen fixing cyanobacterium (*Anabaena azollae*) and capable of increasing rice production when applied as green manure or grown along with rice plant as dual crop [91, 92, 93, 94, 95]. Watanabe (1982) found that the nitrogen fixing ability of each leaf of *Azolla* varies with leaf maturity and the frequency of the heterocyst [96]. Kolhe and Mittra (1990) reported that in rotating rice-wheat cropping system *Azolla* has beneficial effects also on wheat cultivation [97].

Oso (1988) reported that because of the high moisture holding capacity of water hyacinth it can increases the water holding capacity of soil which will promote good adhesion to seed and the coarse powder of the roots can be used as aid in crop production especially vegetables [98]. A study in India by Gunnarsson and Mattsson (1997) showed that decomposition of water hyacinth with sandy clay loam soil and their application in rice cultivation results in enhanced grain production [99]. A report from PACE (2013) explains that composting of dried water hyacinths with soil, ash and organic municipal waste is a low budget option for improving crop yield [100]. According to Malik (2007) the green manure produced by utilizing water hyacinths has very good effect on crop yield in case of potatoes, tomatoes and lady's fingers [81]. Isichei *et al.,* (2003) reported that water hyacinth can be used as fertilizer with other organic waste and also in paper production [101].

In Biogas Production

Aquatic plants is an important component for the water bodies by treating the sewage by biological filtration and also it is the main source of oxygen and carbon dioxide and generate habitats for small fish and other aquatic organism. The problem for water bodies arise when mass invasive occurrence of these plants inhibits the food, sunlight and nutrient supply to other associated plants. By regular harvesting of these plants can be helpful to maintain the water quality. And biogas can be produced from the waste harvested material by anaerobic digestion which will be cheap and pollution free by lower the emission of green house gas. A simple and strong model in excel solver was improved in biogas plant by anaerobic digestion which was the best option to reduce the GHG emission and profitable for the biogas operator by diversifying the income source and protecting environment [102].

Some researches, particularly in the Philippines have investigated on *Azolla* for the production of biogas and found that anaerobic fermentation of *Azolla* or mixture of *Azolla* and rice straw results in the production of methane gas which can be used as fuel. From studies conducted by Newton (1976) it has been noticed that when *Azolla-Anabaena* are grown in a water medium containing nitrate and/or in nitrogen free atmosphere, the nitrogenase in the algal symbiont cannot fix nitrogen, instead it produces hydrogen by using water as the source [103]. Newton (1976) conducted a study on hydrogen production rate of *Azolla* and found that it can produce hydrogen at a rate of 760 nmol H_2/ g^1 fresh weight/hour [103]. According to Hall *et al.,* (1995) the rate of production of hydrogen by *Azolla*

plants can increased by exposure to a microaerobic environment, a partial vacuum or carbon dioxide-enriched or Argon enriched atmospheres or by immobilization of cells of *Anabaena azollae* isolated from the fern [104]. A study by Gopal (1987) describes about the water hyacinth that it can be used as a good source for biogas production as it contains 64% methane of its biomass [105]. Höglöv (2000) reported that we can produce biogas from water hyacinths by anaerobic process after adding other organic substrates with it [106]. Bhattacharya and Kumar (2010) observed that water hyacinth have high nitrogen concentration and can be utilized for biogas production in combination with cow dung [107]. Pantawong *et al*., (2015) conducted a test and found that water lettuce (*Pistia stratiotes*) contains maximum amount of methane as compared to other aquatic weeds like *Eichhornia*, *Salvinia* and *Lemna* [11]. They concluded on the point that water lettuce can be a suitable substrate for biogas production in future with some upscale studies. Kumari *et al*., (2017) reported that due high biomass productivity and rapid growth rate, species of *Salvinia* can be used in biofuel production, especially in the production of bio-ethanol and bio-methanol [108].

In Paper Production

According to Thyagarajan (1983) medium quality paper/boards such as cardboard and coloured cards/cover papers can be made from the pulped stalk of water hyacinth (*Eichhornia crasipes*) [109].

In Mosquito Control

Azolla can also be utilized in mosquito control. Ansari and Sharma (1991) have conducted a survey of ponds, pools, wells, rice fields and drains and found that the water bodies which were completely covered with *Azolla* were almost completely suppressing the breeding of *Anopheles* spp. mosquito and the breeding of *Culex* spp. was not completely inhibited but was reduced [110]. Rajendran and Reuben in 1988 conducted a study in laboratory condition and found that *Azolla pinnata* has great potential in suppressing adult emergence of *Culex quinquefasciatus* Say, and *Anopheles culicifacies* Giles, but not larval survival [111]. Rajendran and Reuben (1991) found a significant decrease in the immature mosquito population of *Anopheles subpictus* Grassi, *Culex pseudovishnui* Colles, and *C. tritaeniorhynchus* Giles, up to 90%, where the water bodies covered with a mat of *Azolla microphylla* Kaulf [112].

CONCLUSION

Normally the free-floating hydrophytes are regarded as invasive and unwanted weeds in water resources as they create problem in normal human usages, irrigation, drainage, etc. On the other hand these hydrophytes can be used as an alternative source of food, fodder, medicine, fertilizer, water purifier and also in biogas or biofuel production, paper production, mosquito control. The hydrophytes are used for treatment of a range of diseases including dysentery and hepatic disorders. Now-a-days when mankind is threatened by drastic global environmental changes triggered by its own activities, we need to investigate and develop alternative ways for conducting our affairs. This paper has just tried to bring together some past and recent findings with future aspects related to the usages of free floating hydrophytes. Further collaborative efforts in research works are required to know the better uses of these useful aquatic resources.

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Fig. 1. *Azolla pinnata* R.Br

Fig. 2. *Eichhornia crassipes* (Mart.) Solms

Fig. 3. *Pistia stratiotes* L.

Fig. 4. *Salvinia molesta* D. Mitch

Fig. 5. *Salvinia cucullata* Roxb. ex Bory

Fig. 6. *Lemna minor* L.

Fig. 7. *Spirodela polyrhiza* (L.) Schleid.

Fig. 8. *Wolffia globosa* (Roxb.) Hartog & Plas

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RESEARCH ARTICLE

A Study on Diversity of Lichen in the North-West Transitional Zone of Mayurbhanj District of Odisha, India

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ABSTRACT

The present field study in transitional zone of Similipal Biosphere Reserve in Mayurbhanj district of Odisha revealed a total 32 numbers species belongs to 24 genus and 11 families. The survey mostly covers the north and west part of the Biosphere Reserve taking 5 different locations (Jashipur, Jamuani, Kaliani, Pantho, Karanjia). Although these area mostly dominated by crustose form of lichen, but they are also enriched in foliose form, which indicate the ecology and air quality level in these areas. Graphidaceae is the dominant family representing 7 numbers of species followed by Caliciaceae representing 6 species. Teloschistaceae, Chrysothricaceae, Lecanoraceae and Letrouitiaceae are represented by single species each. This study area is mostly dominated by trees like *Shorea robusta* Gaertn. followed by *Mangifera indica* L. and *Simarouba glauca* DC. provided suitable conditions for luxuriant growth of lichens. Out of 34 species, 27 species are crustose type and rest 7 are foliose form.

Keywords: Lichen diversity, Similipal Biosphere Reserve, Mayurbhanj, Odisha

INTRODUCTION

Lichens are symbiotic consortium of algae and fungi. The diversity of lichen is widely spread i.e. they are most common in every ecosystem as their peculiar and physiological structure tolerates extreme abiotic stress like cold, and drought condition [1]. However, during last decade due to developmental activities such as urbanization and industrialization results in deterioration of environment quality, which causes decrease in diversity and distribution of the lichen species [2]. India is considered as one of the mega-diversity countries of the world. About 2303 species of lichens are reported in the temperate, alpine, subtropical and tropical region of India. This 2303 species belongs to 305 genera and 74 families [3]. In Odisha lichen diversity was studied in different localities, which includes Jharsuguda district [4], Bhitarkanika National Park [5], Kapilash Reserve Forest [6] and Sun temple of Konark [7].

The lichen taxa enumerated in earlier studies were compiled by some researchers and reported occurrence of 252 species belonging to 81 genera and 35 families [8]. Another study also reveals the occurrence of bio-deteriorating lichen species belonging to genera of *Lecidella*, *Buellia*, *Lecanora* and *Lepraria*, growing luxuriantly on historical monuments of Ratnagiri and Udayagiri in Jajpur district [9].

Mayurbhanj is the largest district in the state of Odisha according to the area coverage, which is dominated by dry deciduous forest. The climate of this region is very much suitable for growth of diverse group of plants including lichens. The lichen diversity of the region has been poorly known as compared to other areas of the state. Although researcher have explored some areas of the district and reported [4] species of lichen, but all the localities explored are confined [10] in the area of Similipal Biosphere Reserve. In the present study, some unexplored localities such as Jamuani, Kaliani, Karanjia, Pantho, Tulasibani situated in the north-west part of the district were covered for the lichen diversity study and results are reported here.

MATERIALS AND METHODS

Study site

The western part of Mayurbhanj district is mainly comprises of long hill range and deep Sal forest. The localities explored in the study (Jamuani, Kaliani, Karanjia, Pantho, and Tulasibani) are situated in north-western part of the district (Fig.1).

Method

More than 120 lichen specimens were collected from the area under investigation in the district of Mayurbhanj. The specimens were identified on the basis of their morphological, anatomical characteristics as well as chemistry following the available literature [11, 12, 13, 14, 15]. The colour tests were carried out with aqueous potassium hydroxide (K), Steiner`s stable paraphenylenediamine (PD) and aqueous calcium hypochlorite (C). The identified samples were deposited in the 'Lichen Herbarium' of CSIR-National Botanical Research Institute, Lucknow (LWG).

RESULTS AND DISCUSSION

The identification of the collected voucher specimens revealed the occurrence of 34 species belonging to 24 genera and 11 families (Table 1). The study area shows the dominance of crustose lichens (27 species) followed by foliose lichens (7 species). Graphidaceae is the dominant family represented by 7 species belonging to 5 genera, followed by Caliciaceae with 6 species of 5 genera, Trypetheliaceae with 5 species of 3 genera, Parmeliaceae with 4 species of 2 genera, Arthoniaceae with 4 species of 3 genera, Ramalinaceae with 2 species of 1 genera, Pyrenulaceae with 2 species of 2 genera and Teloschistaceae, Chrysothricaceae, Lecanoraceae, Letrouitiaceae each with 1 species. It is revealed from the Figure-2 that Graphidaceae is the most dominant family in the north-west site of Similipal which comprises of seven species while other two dominant families of the area are Caliciaceae and Trypetheliaceae with 6 and 5 species respectively.

CONCLUSION

The survey clearly indicated that the North-West region of Similipal Biosphere Reserve is primarily dominated by the varieties of foliose lichen, which is an indication of less anthropogenic activity and low pollution level. The species of *Parmotrema* and *Dirinaria* are observed to be the common foliose species in the localities. Apart from the foliose form the crustose lichens like *Sarcographa* and *Graphis* are the most abundantly occurring species. The present study will be helpful in understanding the ecology of the local forest and shall throw some light to develop future strategies for forest conservation programme.

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Table 1: Lichen species diversity in different selected localities of North-East Similipal Biosphere Reserve, Odisha.

Sl. No.	Lichen Taxa	Family	Study sites				
Crustose form			**A**	**B**	**C**	**D**	**E**
1.	*Amandinea submontana* Marbach.	Caliciaceae	**+**	**+**	**-**	**+**	**-**
2.	*Anthracothecium thwaitesii* (Leight.) Müll. Arg.	Pyrenulaceae	-	+	+	-	+
3.	*Astrothelium indicum* (Upreti & A. Singh) Aptroot & Lucking.	Trypetheliaceae	+	-	+	+	-
4.	*Arthonia medusula* (Pers.) Nyl.	Arthoniaceae	+	-	-	-	+
5.	*Bacidia convexula* (Mull. Arg.) Zahlbr.	Ramalinaceae	+	+	-	-	+
6.	*Bacidia incongruens* (Stirt.) Zahlbr.	Ramalinaceae	**-**	**+**	**+**	**-**	**-**

7.	*Buellia stellulata* (Taylor) Mudd.	Caliciaceae	**+**	**-**	**-**	**-**	**+**
8.	*Caloplaca aurantia* (Pers.) Hellb.	Teloschistaceae	**+**	**-**	**+**	**+**	**-**
9.	*Chrysothrix candelaris* (L.) J.R. Laundon	Chrysothricaceae	**+**	**+**	**-**	**+**	**+**
10.	*Cratiria obscurior* (Stirt.) Marbach & Kalb.	Caliciaceae	-	+	+	-	-
11.	*Cryptothecia bengalensis* Jagadeesh & al.	Arthoniaceae	+	-	-	+	+
12.	*Diorygma junghuhnii* (Mont. & Bosch.) Kalb. & al.	Graphidaceae	-	+	+	-	-
13.	*Dyplolabia afzeli* (Ach.) A. Massal.	Graphidaceae	**+**	**+**	**-**	**-**	**-**
14.	*Graphis filiformis* Adaw. & Makhija	Graphidaceae	**-**	**+**	**-**	**+**	**+**
15.	*Graphis scripta* (L.) Ach.	Graphidaceae	**+**	**+**	**+**	**+**	**+**
16.	*Graphis tenella* Ach.	Graphidaceae	**-**	**+**	**+**	**+**	**-**
17.	*Lacanora achroa* Nyl.	Lecanoraceae	+	+	-	-	-
18.	*Letrouitia transgrassa* (Malme) Haf. & Bellem.	Letrouitiaceae	+	+	-	-	-
19.	*Nigrovothelium bullatum* Lucking, Upreti & Lumbsch.	Trypetheliaceae	+	-	-	-	+
20.	*Nigrovothelium tropicum* (Ach.) Lucking, M.P. Nelsen & Aptroot.	Trypetheliaceae	+	+	+	-	-
21.	*Pyrenula leucotrypa* (Nyl.) Upreti.	Pyrenulaceae	-	+	-	-	+
22.	*Sarcographa maculosa* Zahlbr.	Graphidaceae	**-**	**+**	**-**	**-**	**-**
23.	*Sarcographa tricosa* (Ach.) Mull. Arg.	Graphidaceae	**+**	**-**	**+**	**+**	**-**
24.	*Trypethelium eluteriae* Spreng.	Trypetheliaceae	**-**	**+**	**-**	**+**	**+**
25.	*Trypethelium tropicum* (Ach.) Müll. Arg.	Trypetheliaceae	**+**	**-**	**-**	**+**	**-**
26.	*Tylophoron nidulans* Stirt.	Arthoniaceae	**+**	**-**	**+**	**-**	**-**
27.	*Tylophoron protrudens* Nyl.	Arthoniaceae	**-**	**+**	**+**	**+**	**-**
Foliose form							
28.	*Bulbothrix isidiza* (Nyl.) Hale.	Parmeliaceae	**+**	**-**	**-**	**-**	**+**
29.	*Dirinaria aegialita* (Afzel. ex Ach.) B.J. Moore.	Caliciaceae	-	+	+	-	-
30.	*Dirinaria applanata* D.D. Awasthi.	Caliciaceae	-	+	-	-	-
31.	*Parmotrema praesorediosum* (Nyl.) Hale.	Parmeliaceae	+	+	-	+	-
32.	*Parmotrema raticulatum* (Taylor) M. Choisy.	Parmeliaceae	-	-	+	-	-
33.	*Parmotrema tinctorum* (Despr. ex. Nyl.) Hale.	Parmeliaceae	+	+	-	+	-
34.	*Pyxine cocoes* (Sw.) Nyl.	Caliciaceae	+	+	-	-	-

Note: + Present, - Absent, A- Jamuani, B- Kaliani, C- Tulasibani, D- Pantho, E- Karanjia

(STUDY SITES) **(MAYURBHANJ)**

Figure 1: Study site representing north-west side of Similipal Biosphere Reserve

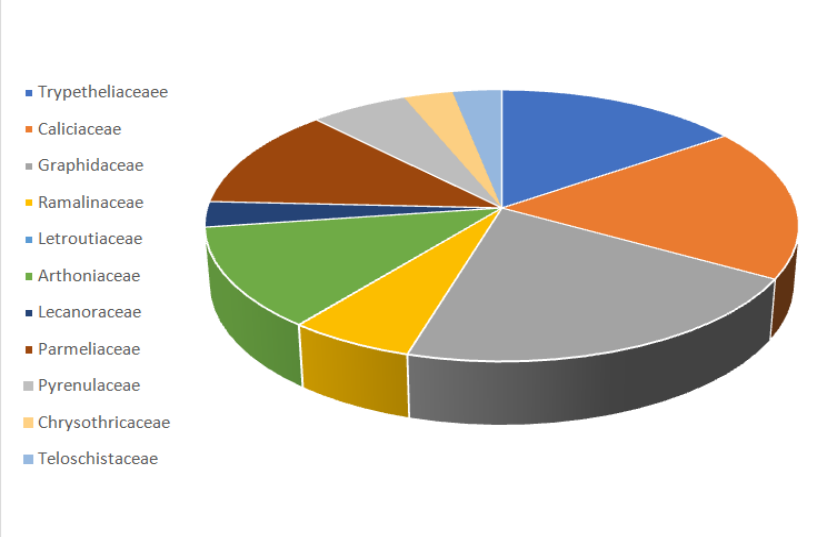

Figure 2: Species abundance (family-wise)

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RESEARCH ARTICLE

Study of Haematological Parameters of Fresh Water Fishes (*Clarias batrachus* and *Pangasius hypophthalmus*) and Marine Fishes (*Mugil cephalus* and *Parastromateus niger*)

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ABSTRACT

The aim of the present study was to obtain a basic knowledge of the haematology of freshwater fishes and marine fishes. Haematological parameters (RBC, WBC, Hb, PCV, MCV, and MCHC) were measured. RBC counts obtained for *Clarias batrachus, Pangasius hypophthalmus, Mugil cephalus* and *Parastromateus niger* were 2.879 ± 0.279×10^6/mm^3, 3.99 ± 0.834×10^6/mm^3, 2.728 ± 0.495×10^6/mm^3, 1.202 ± 0.346×10^6/mm^3 respectively, mean of the WBCwere 7.905 ± 1.058×10^3/mm^3, 11.30 ± 1.790×10^3/mm^3, 12.530 ± 1.410 ×10^3/mm^3, 9.730 ± 1.558×10^3/mm^3 respectively, mean of the Haemoglobin (Hb) were 9.510 ± 1.042g/dl, 9.040 ± 1.896g/dl, 8.820 ± 1.241g/dl, 10.690 ± 1.228g/dl respectively, mean of PCV were 32.5 ± 2.410%, 37.6 ± 4.473%, 37.6 ± 9.274%, 27.9 ± 4.290% respectively. Notable differences were observed in mean ofMCV was 116.607± 12.956fl, 94.235 ± 14.270fl, 138.235± 14.270fl, 232.5± 39.734fl respectively, mean of MCHi.e., 33.032± 3.855pg, 22.656 ± 4.188pg, 32.426 ± 10.536pg, 89.083 ± 24.427pg respectively and mean of MCHCi.e., 28.327 ± 3.156%, 24.042 ± 4.615%, 23.457± 6.500% and 38.315 ± 9.047% respectively. Variation were observed in the data obtained from the analysis of parameters in four species (freshwater and marine) considered. Significant differences observed in some haematological indices like MCV, MCH, and MCHC between the fishes indicate that this inquiry may be helpful as a tool to monitor the health stature of these and other related species. The evaluation of haematological parameters will grant early detection of clinical pathology as well as the presence of disturbance in the aqueous environment.

Keywords: Haematology, Marine fish, Freshwater fish

INTRODUCTION

Consumable fishes are roughly divided into two categories, under the influence of the ecosystems they habituate. The salinity of the water being an important measure, there exist freshwater and marine fishes. The marine fishes are accustomed to a rather saline habitat, hence are endowed with the physiology to be able to survive in higher salt conditions. The bony fishes swallow the salty water to make up for the water lost, the cartilaginous fishes on the other hand increase the concentration of their blood to be in sync with the concentration of sea water. The freshwater fishes are suited to a rather hypotonic environment and they osmoregulate by managing the solute composition of their fluids and dilute urine (Thorson et al. 1967; Thorson, et al. 1973). Fishes are cultured in order to add to the diet enrichment that humans require in their day to day lives. Haematological assessment is done and parameters are considered for an indication of fish health and if their metabolisms are in proper form (De Pedro et al. 2001). The analysis can provide insight on the living conditions of the fish (Bahmani et al. 2001). In the study, we have taken four fish species which are bony and cartilaginous and apart from their physiological differences, they belong to marine and fresh water habitats. To know about the fish health, we need to have standard indices of values that can compare to fishes where exogenous factors might affect their being (Svobodova et al. 2008). The purpose of this analysis is to find those values and throw light on how these indices differ from species to and habitat to habitat, thus giving a datum for future comparison as well. It would also be helpful in comparing the impact of different habitat on fish community.

MATERIAL AND METHODS

The experiment was carried out at the Department of Zoology, of Centurion University of Technology and Management in Jatani (Bhubaneswar campus) for the haematological assessment of the various piscine species. Sampling. An aggregate number of 80 fishes were used in the experiment for the haematological assessment. Healthy fishes of four species namely, *Pangasius hypophthalmus*, *Clarias batrachus, Mugil cephalus* and *Parastromateus Niger*were collected from the fish breeders of Jatani and Chilika. The fish samples were collected between October 2019 to December 2019. The fishes collected were devoid of any external injury or morphological defects. The average body weight of the fishes differed, depending on their species and the body length ranged from 35 cm to 50cm.
Twenty fishes of each species were collected for the purpose of this study.

Haematological analysis

The blood sample was collected from the fish, with the help of a single use Dispo van syringe. The procedure was repeated for all twenty fishes of all four species. The blood was transferred into an EDTA vial and proceeded for immediate haematological assessment. The RBC and WBC were counted after proper dilution with Hayem's and Turk's fluid respectively. An improved Neubauer's haemocytometer was used. The Sahli's haemoglobinometer was used for determination of Haemoglobin content of the blood sample. Packed cell volume (PCV) was calculated with the use of a Wintrobe tube. The Mean Corpuscular Volume (MCV), the Mean Cell Haemoglobin (MCH), and the Mean Cell Haemoglobin Concentration (MCHC) were calibrated from the previous values.

STATISTICAL ANALYSIS

Table 1: Haematological Parameters of Freshwater and Marine Fishes

RESULT AND DISCUSSION

The haematological values obtained, are mentioned in the given table. Considerable variations can be spotted between the diverse species considered. The highest level of RBC was expressed in the *Pangasius hypophthalmus*

(3.99±0.834×10^6/mm^3), indicating an increased oxygen demand in the fish as compared to the other species. The descending RBC record added *Clarias batrachus* (2.879±0.279×10^6/mm^3)to the list, followed by *Mugil cephalus* (2.728 ± 0.495×10^6/mm^3) and lastly *Parastromateus niger* (1.202 ± 0.346×10^6/mm^3)with the lowest count. In the present study, the RBC count of *Pangasius hypophthalmus*was estimated as 3.99±0.834×10^6/mm^3 which is slightly more than the values reported by Yaghobi et al. (2013), who noted the RBC value in *Pangasius hypophthalmus*as 3.05±0.55×10^6/mm^3. The RBC value of *Mugil cephalus*, 2.728 ± 0.495×10^6/mm^{3}was slightly less than the values obtained by Faggio et al. (2014) which was 3.53±0.16×10^6/mm^3. The value obtained for *Clarias batrachus* was 2.879±0.279×10^6/mm^3 which is in agreement with the findings of Acharya et al. (2014) who opined it to be 2.97±0.07×10^6/mm^3.

The WBC value was recorded to be significantly highest in the *Mugil* cephalus (12.530 ± 1.410 ×10^3/mm^3) which, followed by *Pangasius hypophthalmus*(11.30 ± 1.790×10^3/mm^3), with *Parastromateus niger*(9.730 ± 1.558×10^3/mm^3) and *Clarias batrachus* (7.905 ± 1.058×10^3/mm^3) finishing last. The elevated levels of WBC might point towards high immunity in the *Mugil cephalus* owing to it marine habitat, or exposure to an infection that might have hiked up the count. The WBC value of Mugil*cephalus*was12.530 ± 1.410 ×10^3/mm^{3}which is less than the values recorded by Faggio et al. (2014) which was 18.30±0.40 ×10^3/mm^3. The WBC value of *Pangasius hypophthalmus*was 11.30 ± 1.790×10^3/mm^3 which close to the values reported by Yaghobi et al. (2013), which was 12.37±1.6×10^3/mm^3. *Clarias batrachus* has a WBC value of 7.905 ± 1.058×10^3/mm^{3}which is in agreement with the findings of Acharya et al. (2014) who opined it to be 7.63±0.35×10^3/mm^3.

The Haemoglobin (Hb) values in the present study were observed to be significantly higher in *Parastromateus niger* and lower WBC values were noted in *Pangasius hypophthalmus*, *Clarias batrachus* and *Mugil cephalus* in a descending fashion. The Hb value of *Pangasius hypophthalmus* was9.040 ± 1.896g/dl which is lower than the value mentioned by Yaghobi et al. (2013), where the Hb value is 13.28 ±0.25g/dl. The Hb value of *Clarias batrachus* was 9.510 ± 1.042g/dl which is in agreement with the findings of Acharya et al. (2014) who opined it to be 9.5±0.31g/dl. The Hb value of *Mugil cephalus* was 8.820 ± 1.241g/dl which was lower than the Hb value 10.65±0.60 mentioned by Faggio et al. (2014). The low Hb levels can be associated with the diminished activity in the fishes thus relating it with a low requirement of oxygen, binding to which serves as the prime purpose of the compound.

The Mean Corpuscular Haemoglobin (MCH) differed accordingly, as the value of it depends on the Hb data. The highest MCH values was recorded in *Parastromateus niger* which was 89.083±24.427pg. Next the MCH value of *Clarias batrachus* was 33.032 ± 3.855pg which is close to the value noted by Acharya et al. (2014), 33.25±0.74. It was followed by the MCH value of *Mugil cephalus* which was 32.426±10.536pg, it was slightly more than the 29.89±0.60 value mentioned by Faggio et al. (2014). The lowest MCH value was recorded in *Pangasius hypophthalmus*, 22.656 ±4.188pg.
The Packed Cell Volume (PCV), which is an estimate of the corpuscular volume of the blood was found to be highest in *Pangasius hypophthalmus*(37.6 ± 4.473%) and *Mugil cephalus*(37.6 ± 9.274%), lowest in *Parastromateus niger*(27.9 ± 4.290%) and intermediate in *Clarias batrachus* (32.5 ± 2.410%).The Mean Cell Volume(MCV), which is relative to the PCV followed suit and the differential was noted to be in a dissimilar manner, with *Clarias batrachus*(232.5 ± 12.956fl), at highest, followed by *Mugil cephalus*(138.235 ± 14.270fl), holding *Parastromateus niger*(116.607 ± 39.734fl) below it and *Pangasius hypophthalmus*(94.235 ± 14.270fl)at the bottom. The Mean Corpuscular Haemoglobin Concentration (MCHC) which was calculated from the values of MCH and MCV had *Parastromateus niger* (38.315 ± 9.047%) at the top, with *Clarias batrachus* (28.327 ± 3.156%),*Pangasius hypophthalmus* (24.042 ± 4.615%),and *Mugil cephalus* (23.457 ± 6.500%)in a descending format.

CONCLUSION

The assessment of haematological characters have been a fruitful approach to impose judgment regarding the well-being of the fish. If the haematological values are extremely deviated from the normal course, there can be efforts to find the reason behind the anomaly and thus determine the changes concerned. Under certain circumstances, the different in biochemical parameters of the water, a decrease in the availability of viable living conditions such as

prey, spawning grounds etc. can be spotted early and the species can be saved from loss. The fish haematology can be deemed as extremely significant because there are still fishes who haven't been considered for research and ignoring the fact whether they are consumable or not, they do contribute their part for ecological balance. Further research in this field will encourage conservation of those fishes as their health status will be easier to determine. After finding out all the values, it was concluded that the saline and freshwater habitat indicate different haematological values in the species considered to ensure their sustainability.

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Table 1: Haematological Parameters of Freshwater and Marine Fishes

	Clarias batrachus	*Pangasius hypophthalmus*	*Mugil cephalus*	*Parastromateus niger*
RBC($\times10^6$/mm^3)	2.879 ± 0.279$\times10^6$/mm^3	3.99 ± 0.834$\times10^6$/mm^3	2.728 ± 0.495$\times10^6$/mm^3	1.202 ± 0.346$\times10^6$/mm^3
WBC($\times10^3$/mm^3)	7.905 ± 1.058$\times10^3$/mm^3	11.30 ± 1.790$\times10^3$/mm^3	12.530 ± 1.410 $\times10^3$/mm^3	9.730 ± 1.558$\times10^3$/mm^3
Hb(g/dl)	9.510 ± 1.042g/dl	9.040 ± 1.896g/dl	8.820 ± 1.241g/dl	10.690 ± 1.228g/dl
PCV (%)	32.5 ± 2.410%	37.6 ± 4.473%	37.6 ± 9.274%	27.9 ± 4.290%
MCV(fl)	116.607 ± 12.956fl	94.235 ± 14.270fl	138.235 ± 14.270fl	232.5 ± 39.734fl
MCH(pg)	33.032 ± 3.855pg	22.656 ± 4.188pg	32.426 ± 10.536pg	89.083 ± 24.427pg
MCHC (%)	28.327 ± 3.156%	24.042 ± 4.615%	23.457 ± 6.500%	38.315 ± 9.047%

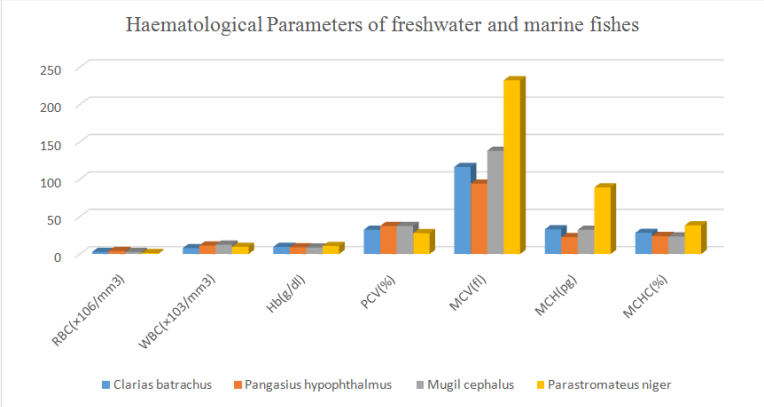

Fig.1. Haematological Parameters of freshwater and marine fishes

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RESEARCH ARTICLE

Antimicrobial Efficacy of *Azadirachta indica* against *Enterococcus faecalis* and *Candida albicans* – An *In vitro* Study

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ABSTRACT

To compare the Antimicrobial efficacy of neem extract as intracanal medicament when compared with calcium hydroxide. Neem leaf extract and calcium hydroxide was used to assess the antimicrobial efficiency. Agar well diffusion test was used to study the antimicrobial efficacy. Each well was loaded with 20, 40, 60, 80 and 100 µl of corresponding concentration of neem extract and calcium hydroxide was used. The plates were incubated for 24h at 37°C. The development of inhibition zone around the well was measured (diameter). The zone of inhibition was recorded, tabulated, and analyzed statistically with the help of IBM Statistical Package for the Social Sciences statistics version 20 using analysis of variance test. The neem extract samples were subjected to antibacterial activity (MIC & MBC) by micro dilution method against *Entercoccus faecalis* and *Candida albicans.* Wells containing concentrations of sample that showed growth were said to have minimum inhibitory concentration, while wells with least concentration of sample that showed no growth were regarded as minimum bactericidal concentration, MBC. Both the medicaments showed well-defined and comparable zones of inhibition around their respective wells. Zone of inhibition of neem extract was significantly higher than calcium hydroxide. Analysis of variance showed significant difference between zone diameters of neem leaf extract, and calcium hydroxide against *E. faecalis and C.albicans* ($p < 0.05$). From the present study, it can be concluded that neem leaf extract shows significant difference in zones of inhibition when compared with calcium hydroxide.

Keywords: Neem extract, Antimicrobial activity, Inhibition zone, MIC, MBC

INTRODUCTION

Eradication of bacteria from the root canal system is important in ensuring the long-term success of root canal therapy. The microorganisms are the primary etiological agent in endodontic infections, and failure to eradicate them affects the outcome of endodontic therapy (1). Studies have shown that the bacterial flora in endodontic infections is polymicrobial, with a predominance of anaerobic species (2). This is mainly achieved through the reduction of bacteria by debridement of the root canal system utilizing mechanical preparation of the canals combined with chemical interventions. Several areas of the root canal walls, specifically in the apical third, are difficult to clean mechanically (3).

Enterococcus faecalis is part of the human normal flora and an important pathogen in opportunistic infections in humans (4). *E. faecalis* is rarely present in primary apical periodontitis, but it is the dominant microorganism in root canal-treated teeth presenting with posttreatment apical periodontitis (5). Eradication of *E. faecalis* from the root canal remains a challenge, since it is resistant to a variety of antimicrobial agents.Candida albicans is the most common species isolated from the root canal (6).The resistance to calcium hydroxide and ability to penetrate into side canals and dentinal tubules are possible reasons for the occurrence of oral Candida species in microflora of persistent apical periodontitis (7, 8). The yeasts in the infected root canal system may cause persistent apical periodontitis (Nair et al. 1990, Waltimo et al. 1997).The control and suppression of *E. faecalis* and *C.albicans* in these dental procedures are of primarily importance in decreasing the penetration of bacteria inside the dentinal tubules and also limiting the formation of any relationship with other microorganisms, as in virulence factors, environment, and the biofilms (9).

Azadirachta indica (neem) is known for its Indian medicinal value. Several parts of the plant such as fruits, seeds, leaves, and bark are used to isolate more than 140 compounds (10). The isoprenoid group of neem leaf and its constituents has demonstrated anti-inflammatory, immune-modulatory, antibacterial, antifungal, antiviral, antioxidant, and anti-carcinogenic properties (11). Neem is considered to be a "village dispensary" since every part of the tree including the leaves, bark, and seeds have medicinal properties (12). US National Academy of Sciences recognized the importance of neem tree in 1992 and entitled neem as "a tree for solving global problems" (13). Although initial scientific reports have demonstrated favorable *in vitro* results, there is no *in vivo* study that has compared the antibacterial efficacy of the herbal extracts as intracanal medicament. The aim of the present *in vitro* study is to evaluate and compare the antimicrobial efficacy of *Azadirachta indica*, and calcium hydroxide as intracanal medicaments. The null hypothesis to be tested was that there was a difference in the antimicrobial efficacy of *Azadirachta indica*, and calcium hydroxide as intracanal medicament.

MATERIALS AND METHODS

In this study *E. faecalis* and *C.albicans* species was used to test the antimicrobial efficacy. The bacterial culture was prepared in sterile Luria broth and adjusted to an optical density to match the turbidity of a McFarland 0.5 scale. The sensitivity assay was done using Neem leaf (*A. indica*) extract and Calcium Hydroxide (RC Cal).

Preparation of Neem Extract

Neem leaves were shade dried, powdered and stored in air-tight containers. They were soaked at room temperature for 24 hours, and then filtered. Air dried Neem powder was repeatedly macerated with 500ml of 99% ethanol and filtered using whatman filter paper. The ethanol was evaporated and the extracts were concentrated using rotary flash evaporator and used in the assay (14, 15).

Agar Well Diffusion Method

Sample Preparation: 10mg/ml of sample was provided for antimicrobial assay.

Procedure: Luria broth Agar (LBA) plates were inoculated with test organisms. The plates were evenly spread out. Then wells were prepared in the plates with a cork borer. Each well was loaded with 20, 40, 60, 80 and 100 µl of corresponding concentration of neem extract and calcium hydroxide was used. The plates were incubated for 24h at 37°C. The development of inhibition zone around the well was measured (diameter) and recorded. Recording of the zones of inhibition was done for all the culture plates and the results were analyzed statistically with the help of IBM Statistical Package for the Social Sciences statistics version 20 using analysis of variance test.

MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The neem extract samples were subjected to antibacterial activity by micro dilution method against *Entercoccus faecalis* and *Candida albicans.* Luria broth (Himedia, Mumbai) was prepared and sterilized by autoclaving at 121°C, 15 lbs. for 15 minutes. 100µl of broth was added to the 96 well micro titre plates. The 100µl of the given sample was added in the first well and then serially diluted till the eighth well. The 10µl of log phase culture was introduced into the respective wells. Similarly tetracycline (100µl from10mg/ml) was added to 100µl of broth and serially diluted. Then 10µl of log phase culture was added. This served as the positive control. Broth and culture was taken as Negative control. Sterile broth serves as a control. The plates were incubated at 37°C for 24 h and hereafter observed for growth or turbidity. Subsequently, a loopful of broth from each well not showing growth, was inoculated into nutrient agar plate. Thereafter, equal volumes of sterile nutrient broth were added into the wells and incubated further for 24 h at 37°C. Then, the micro titre plate and agar plates were examined for growth or turbidity.

Wells containing concentrations of sample that showed no bacterial growth or turbidity after the first 24 h incubation but showed growth (on agar plate) or turbidity (in well) after the addition of equal volumes of sterile nutrient broth and further 24 h incubation, were said to have minimum inhibitory concentration, while wells with least concentration of sample that showed no growth or turbidity after the first 24 h and still no growth (on agar plate) or turbidity (in wells) after further 24 h (that is, after the addition of equal volume of sterile nutrient broth), were regarded as minimum bactericidal concentration, MBC.

RESULTS

Both the medicaments showed well-defined zones of inhibition around their respective wells (Fig. 1 and 2). Zone of inhibition against *E. faecalis and C.albicans*was more for Neem extract when compared to calcium hydroxide (Table 1 and 2). Variance analysis showed significant difference between zone diametersof neem extract and calcium hydroxide against *E. faecalis and C.albicans* ($p < 0.05$). The minimum Inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Neem Extract was tabulated (Table 3).

DISCUSSION

E.faecalis species is the most common bacterial species to be isolated from the root canal treatment failed teeth (16). Because of this reason it is been tested against in this present study. Because of the therapeutic properties these herbal medicaments are becoming popular in dentistry and also in other fields of medicine. This present study evaluated the antimicrobial efficacy of neem extract as intracanal medicament in comparison with calcium hydroxide. The present study showed more inhibitory mean zone of 28.6 mm for Neem extract followed by calcium hydroxide [Table 1 and Figure 1]. This result is in favor of the study by Vinothkumar *et al*. who observed neem to be highly effective against *E. faecalis* and *Candida albicans.*

Calcium hydroxide is the most widely used intracanal medicament, requiring a disinfection period of 7 days.The high pH of calcium hydroxide formulations alters the biologic properties of bacterial lipopolysaccharides in the cell walls of gram-negative species and inactivates membrane transport mechanisms, resulting in bacterial cell toxicity

(17). So in this study the antimicrobial efficacy of neem extract is compared with calcium hydroxide. Neem (*A. indica* A Juss, margosa tree) is a traditional plant in India, with medicinal properties in each part of the tree. It has antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and immune-stimulatory activity. The isoprenoid group (nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid) of constituents of neem has a broad range of therapeutic and antimicrobial effects suggesting its potential as an endodontic intracanal medicament (18, 19).

The use of neem as an intracanal medicament is advantageous because neem is an excellent antioxidant with a very high biocompatibility, and there is no risk of tissue toxicity with its use. Biocompatibility of neem to the human periodontal ligament fibroblasts has already been proved, and this is an important factor favoring its clinical application in endodontics (20).Because of the presence of nimbidin in neem, it has the bitter taste which is unpleasant for the patient. The bitter tastecan be overcome by adding sweeteners to the neem extract (21).

Bohora *et al*.in 2010 compared the antibacterial efficacy of the neem leaf extract and 2% sodium hypochlorite against *E. faecalis* and *C. albicans* and he found that there was a significant difference between the zone of inhibition of neem leaf extract and the 2% NaOCl against *E. faecalis* and a mixed culture. No significant difference was observed against *C. albicans (*22). Hegde *et al*.in 2013 compared the antibacterial efficacy of 2% sodium hypochlorite, propolis, neem leaf extract, turmeric and liquorice against *E. faecalis* and *C. albicans* and they found that the neem leaf extract showed the highest zone of inhibition against *E. faecalis* and *C. albicans (*23). Ghonmode *et al*.in 2013 evaluated the efficacy of neem leaf extracts, grape seed extracts, 3% sodium hypochlorite, and absolute ethanol, against *E. faecalis* and found that neem leaf extracts showed significantly greater zones of inhibition as compared to the other test materials. So they concluded that the neem leaf extract has a significant antimicrobial effect against *E. faecalis*, commonly found bacteria in the root canal system (24). Dutta et al. in 2014 evaluated the in vivo antimicrobial efficacy of 2.5% sodium hypochlorite and 0.2%chlorhexidinegluconate, and an experimental irrigant from neem tree and concluded that neem had good anti-microbial efficacy and could be considered for endodontic use (25).

Damre et alin 2015 did a study on evaluation of the antimicrobial efficacy of various herbal irrigating agents like neem leaf extract, turmeric, honey, and aloe vera and he observed that honey showed the highest zone of inhibition against *E. faecalis* followed by neem, sodium hypochlorite, turmeric, and aloe-vera (26). Arora et al in 2015 the antimicrobial potential of herbal extracts like neem (Azadirachta indica), tulsi (Ocimum sanctum), bitter gourd (Momordia charantia), and arka (Calotropi sprocera) were used as endodontic irrigants against *E. faecalis* and C. albicans. It was observed that bitter gourd demonstrated the maximum zones of inhibition, followed by neem, tulsi, and calotropis for both *E. faecalis* and *C. albicans* (27). Chandrappa et al. in 2015 assessed the antimicrobial activity of herbal medicines like neem extract, tulsi extract and chlorhexidine and he observed that all of the irrigants expressed significant antibacterial efficiency against *E. faecalis* (28).

Babaji*et al*.in 2016 did an *in vitro* analysis and found that the herbal medicines such as neem, *M. citrifolia*, and *A. vera* showed inhibitory zones against *E. faecalis*. Hence can be used as root canal irrigating solutions (29). Sundaram et al. and Prasad et al. in 2016 evaluated the antimicrobial efficacy of herbal irrigants including neem and they suggested it in the future of endodontics. There is considerable support in the literature that favors using neem as an intracanal irrigant. It should be noted that the present studies have been *in vitro (*30, 31).

CONCLUSION

Under the limitations of the study, it is found that the zone of inhibition in the agar diffusion test showing the antimicrobial efficiency of the neem extract was statistically significant when compared with calcium hydroxide. Therefore, the neem leaf extract could be used as an alternative agent in intracanal medication.

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Table 1: Results for zone of inhibition against *E.faecalis* for Neem extract and Calcium hydroxide

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio (F)	Table value at 0.05 level of Significance
Between columns	12.1	1	12.1	0.34	5.99
Within columns	142.4	4	35.6	2.94	4.53
Total	154.5	6			

Table 2: Results for zone of inhibition against *C.albicans* for Neem extract and Calcium hydroxide

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio(F)	Table value at 0.05 level of Significance
Between columns	12.1	1	12.1	0.23	5.99
Within columns	210	4	52.5	4.34	4.53
Total	222.1	6			

Table 3: MIC and MBC of Neem extract

Organism	Antibacterial Activity	
	Minimum Inhibitory Concentration (μg)	Minimum bactericidal concentration (μg)
E.faecalis	125	250
C.albicans	62.5	125

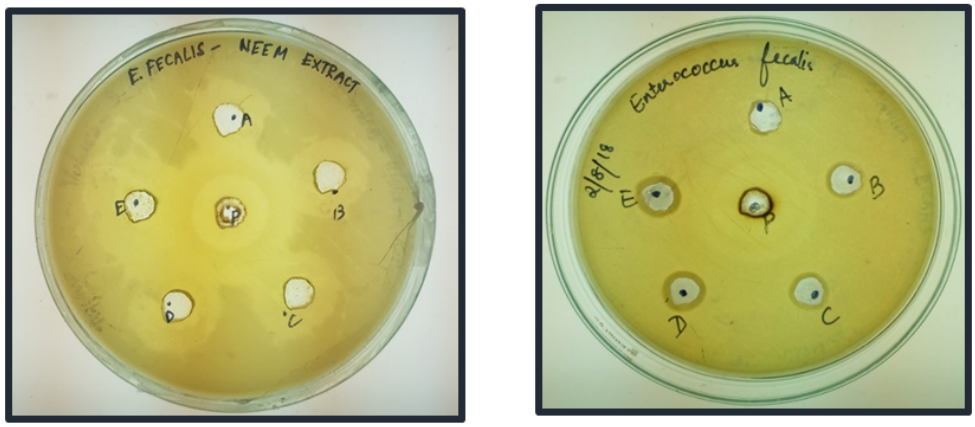

Figure 1: Zone of inhibition for *E.faecalis* for Neem Extract and Calcium Hydroxide

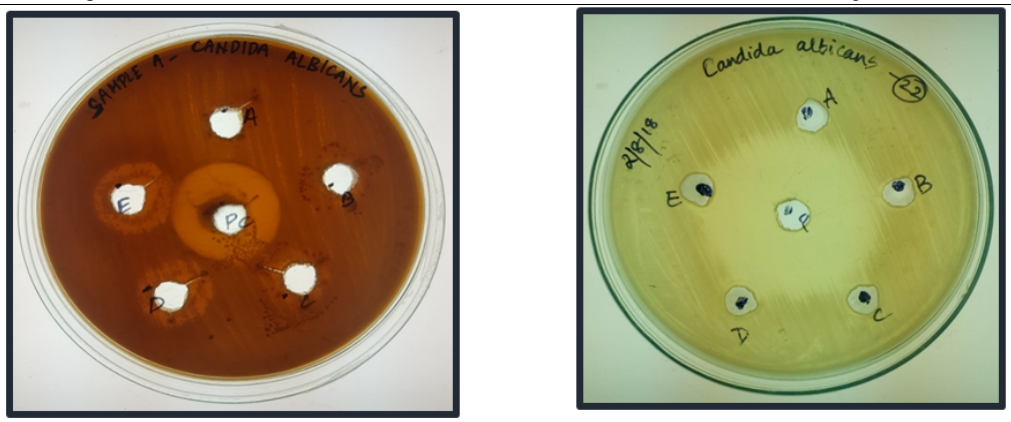

Figure 2: Zone of inhibition for *C.albicans* for Neem Extract and Calcium Hydroxide

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RESEARCH ARTICLE

Lichen: A Potential Source of Phytotherapeutic Agent

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ABSTRACT

Lichens are the symbiotic thallus, which is the result of association between fungi and algae. They play an important role in terms of succession in many ecosystems. They usually produce many unique extracellular secondary metabolites, which are the resultant of symbiosis process. These metabolites are important due to their uniqueness and various pharmaceutical applications. The phytochemical constituents of lichens have several pharmaceutical applications including anticancer, antimicrobial, antioxidant etc, which were being practised by several communities and traditional knowledge holder around the globe since a long period. The present review article describes the importance of lichen bioactive compounds and their pharmaceutical potential.

Keywords: Lichen, Bioactive compound, Pharmaceutical application.

INTRODUCTION

Lichens are the small group of curious plants and nonvascular cryptogams. These are an exclusive group of plant consisting two uncoupled organism, a fungus and an alga or cyanobacterium, growing together in a close symbiotic association and can be seen in most adverse condition of climate and substrate [1]. The unique structure make them more resistant to adverse climatic conditions such as cold or arid condition and hence they are cosmopolitan in nature, as they able to spread in all part of terrestrial world, from equator to polar region in the earth [2]. They also play role as a unique part of the environment, in terms of nutrient cycles and succession [3]. Morphologically, the thallus of lichens can be classified as squamolose, crustose, fruiticose and foliose forms. If the thallus is superficially or entirely developed on the substratum like rocks and barks, it is fructicose type. Usually, lichens contain the photosynthetic partner as algae and the mycobiont is a fungus (Ascomycetes), which help in vegetative or asexual reproduction [4]. Depending upon the substratum on which lichen growth occurs, lichens can be categorized as different types like saxicolous (on rocks), mussicolous (on moss), follicolous (on leaf surfaces), corticolous (on bark of

trees), terricolous (on soil) [5]. Lichens are highly diverse group of plants and because of its cosmopolitan distribution, as they play very important roles in the pioneer of succession and this is the key reason behind the production of large number of metabolites, which give defence against several negative environmental stresses. These metabolites produced by lichens are mostly categorized into two classes i.e. primary and secondary [6, 7]. The primary metabolites which are mostly produced in intracellular mode include carotenoids, amino acids, proteins, vitamins and polysaccharides. However, the most of organic compounds present in lichens are secondary metabolites.

Phytotherapeutic values

As potential antibiotic agents

Lichens manufacture several derivatives like chromones, xanthones, secondary aliphatic acids and esters and anthraquinones [8]. The lichen derivatives are the key substances against various microorganisms. Most investigated lichen secondary metabolites are potential antimicrobial source due to presence of phenolic compounds, triterpenes, steroids, depsides, depsidones and depsones. Some of the most commonly produced acids by lichens are vulpinic acid, usnic acid & stictic acid and are potential source of antimicrobes [9, 10, 11, 12, 13, 14]. Lichen's metabolites are considered as potential source of antimicrobial agents as they have the ability to kill microorganisms or to inhibit their growth and development. The lichen extract and its secondary metabolites are reported for their antimicrobial potential since ancient time [14, 15, 16, 17, 18, 19, 20, 21, 22]. The mechanism by which the bioactive compound of lichen shows its antimicrobial properties may be by inhibition of cell wall synthesis, by degrading the layer of peptidoglycan of bacterial cell wall, inhibition of protein synthesis (Translation), alternating the cell membrane composition, inhibition of the nucleic acid (DNA and RNA) synthesis as well as inhibiting important enzymes.

As potential antioxidant agents

Free radicals or reactive oxygen species (ROS), like hydrogen peroxide and superoxide anion, reactive nitrogen species (RNS) and hydroxyl radical play a major role in several biochemical interactions inside the cell. These reactive nitrogen species (RNS) and reactive oxygen species (ROS) are important at low concentration for the growth of cellular structure and can behave as protection layer for the defense mechanism of cell [23, 24, 25, 26, 27, 28]. The antioxidant defense mechanism is helpful for the balance between the generation of ROS and their reduction under normal circumstance. However, in case of pathogen invasion and unfavourable condition like chilling, salinity, drought, metal toxicity, the ROS are not efficiently removed by the antioxidant defence system and thus the dynamic balance between the diminution of ROS and its generation is broken [29]. Moreover, lichens have good potential of natural antioxidants as they contain hydrophobic secondary metabolites which are mostly phenolic in nature are having good potential of antioxidant activity. As lichens are mostly exposed to unfavourable and stress conditions, hence they possess phenolic compounds which plays vital role in lichen development and growth. May be the up-regulated biosynthetic pathway of lichen bioactive compounds mainly polyphenols, which play crucial role against oxidative stress as they have suitable antioxidant capacity, is due to its exposure to the harsh condition [30]. Phenolic derivatives of lichens are dibenzofurans, depsidones, pulvinic acid derivatives and depsides [17].

Lichen phenolics are mainly dibenzofurans, depsides and depsidones. They usually secreted by the mycobiont and stored as crystals. These lichen-phenolics are majorly acetate-polymalonate-derived; however, pulvinic acid is the only exception. The phenolics of lichen are made up of two monocyclic phenols which bonded by ester bond (depsides) or ether bond (depsidones) or by a furan bond (dibenzofurans) [31]. Excluding phenolic compounds, lichens have been found to possess a number of secondary metabolites with good antioxidant activity like Atranorin, Lecanoric acid, 2,4-Dihydroxy-6-propyl, Barbatic acid, Ramalin, Pannarin, Stictic acid, Divaricatic acid etc [32].

As potential anticancer agents

Lichens synthesize a number of secondary metabolites, many of which are unique to the lichens only. Approximately 1050 secondary compounds have reported till now [33]. This diversity of secondary metabolites mainly possible due to the symbiotic association within the lichen [7]. The compounds are produced mostly by the fungal partner within the lichen [34, 35] and pile up in the cortex. However, a small number of metabolites (50-60) reported from non-lichenized fungi [36]. For instance anthraquinone parietina, which occurs in some fungi and in the vascular plants [37]. Suh et al. in the year 2017 isolated 'ramalin' - a bioactive compound from *Ramalina terebrata* which could be used as a potential anticancer agent [38]. Ramalin basically arrest the cell cycle at G2/M phase which influence the activity of the respective genes, such as cyclin-dependent kinase inhibitor 1A, tumor protein p53, cyclin B1 and cyclin-dependent kinase 1. There are some other evidences where some signaling pathway and RhoGTPase-suppressing activity is affected by bioactive compound which inhibit human lung cancer cells [39]. Somehow, vulpinic acid efficiently inhibited the growth of some treated cell lines [40].

As potential antiviral agents

Lichens have also proven as the source of antiviral bioactive compounds as several investigation in past demonstrated it efficiently, as atranorin exhibited good inhibitory action against virus like hepatitis C [41]. Similarly, Usnic acid derivatives have also efficient antiviral potential against influenza virus [42]. The secondary metabolites of lichen such as hypericin, parietin, lichenan etc. has also proven good source of antiviral agents. For instance, Stubler and Buchenauer 1996 reported metabolites such as lichenan, which is most common among all lichens and had exhibited negative effect on growth of tobacco mosaic virus. Few researchers also explained about antiviral potential of *Nephroma laevigatum*, which contains metabolites like bianthrones, anthraquinones and hypericin [43, 44, 45]. The studies on the screening of 69 species of lichen indicated that *Pseudocyphellaria glabra*, *Pseudocyphellaria homoeophylla* and *Cladia retipora* have potential antiviral properties [46]. The data presented in Table-1 gives an account of important bioactive compounds, their sources and pharmacological activities, which have been investigated by several researchers. The data includes Atranorin, Physodic acid, Protolichesterinic acid, Usnic acid and Zeorin like secondary metabolites, which are responsible for multiple therapeutic applications such as antibacterial, antidiabetic, antifungal, anti-inflammatory, antioxidant, antitumour etc.

CONCLUSIONS

These documented works reveals that the lichens can be an interesting source of bioactive compounds with potential pharmaceutical applications as they contain important secondary metabolites, which are unique to lichens only. The importance of lichenic secondary metabolites could be correlated to their respective therapeutic application from the traditional herbal healers and documented information possessed by different civilizations around the world. The lichens like *Nephroma laevigatum* and *Heterodermia obscura* are considered two important species, as they possess important antiviral metabolites such anthraquinones, bianthrones, and hypericin derivatives. Similarly ralmalin and other secondary metabolites isolated from *Ramalina terebrata*, possesses potential anticancer properties. Further research should be focused on the extraction, isolation and identification of new compounds from lichens and investigation of their phytotherapeutic applications, which could prove beneficial against various diseases.

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Table-1: List of bioactive compounds, their sources and pharmaceutical activity

Bioactive compounds	Source of the lichen taxa	Pharmaceutical activities	References
Alectorialic acid	*Usnea longissima* Ach.	Antiviral	[47]
Atranorin	*Parmelia nepalensis* Tayl., *Parmelia tinctorum* Nyl., *Cladonia foliacea* (Huds.) Willd., *Pseudevernia furfuracea* (L.) Zopf., *Hypogymnia physodes* (L.) Nyl., *Parmelia caperata* (L.) Ach., *Physcia aipolia* (Ehrh.) Hampe., *Umbilicaria polyphylla* (L.) Baumg., *Hypogymnia physodes* (L.) Nyl., *Cladonia kalbii* (*Ahti*) Ahti & DePriest.	Antimicrobial, antioxidant, antiinflammatory, anticancer, antineurodegenerative, antidiabetic	[48, 49, 50, 51, 52, 53, 54]
Barbatic acid	*Cladia aggregata* (Sw.) Nyl.	Antioxidant, antimicrobial	[51, 55, 56]
Benzoic acid	*Ramalina roesleri* Nyl.	Antioxidant	[51, 57]
Biruloquinone	*Cladonia macilenta* Hoffm.	Antineurodegenerative	[51, 58]
Diffractaic acid	*Parmelia nepalensis* Tayl., *Parmelia tinctorum* Nyl.	Analgetic, antiproliferative, antioxidant, antipyretic, analgesic, antiinflammatory	[48, 51, 59, 60, 61, 62]
Divaricatic acid	*Psoroma pallidum* Nyl., *Erioderma chilense* Mont., *Lecanora frustulosa* (Dicks.) Ach., *Parmeliopsis hyperopta* (Ach.) Arnold.	Antioxidant, antimicrobial, antidiabetic	[11, 49, 51, 63, 64]
Divaricatinic acid	*Ramalina farinacea* (L.) Arc.	Antiviral	[51, 65]
Ethyl hematommate	*Parmotrema cooperi* (J. Steiner & Zahlbr.) Sérus.	Antidiabetic	[51, 66]
Ethyl orsellinate	*Parmotrema cooperi* (J. Steiner & Zahlbr.) Sérus.	Antidiabetic	[51, 66]
Evernic acid	*Evernia prunastri* (L.) Ach., *Pseudoevernia furfuraceae* (L.) Zopf.	Antifungal, antibacterial, antioxidant, anticancer	[67, 68]
Fumarprotocetraric acid	*Cladonia foliacea* (Huds.) Willd., *Hypogymnia physodes* (L.) Nyl., *Parmelia caperata* (L.) Ach., *Physcia aipolia* (Ehrh.) Hampe., *Umbilicaria polyphylla* (L.) Baumg.	Antibacterial, antifungal, antioxidant, anticancer, neuroprotective	[69, 70, 71, 72, 73]
Gyrophoric acid	*Hypogymnia physodes* (L.) Nyl., *Parmelia caperata* (L.) Ach., *Physcia aipolia* (Ehrh.) Hampe., *Umbilicaria polyphylla* (L.) Baumg., *Parmotrema cooperi* (J. Steiner & Zahlbr.) Sérus., *Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw.	Antimicrobial, anticancer, antioxidant, antidiabetic	[51, 70, 71, 72 74, 75]
Homosekikaic acid	*Ramalina roesleri* Nyl.	Antioxidant, antibacterial	[57]
Homosekikaic acid	*Ramalina farinacea* (L.) Arc.,	Antiviral	[49]

Lecanoric acid	*Hypogymnia physodes* (L.) Nyl., *Parmelia caperata* (L.) Ach., *Parmotrema cooperi* (J. Steiner & Zahlbr.) Sérus., *Physcia aipolia* (Ehrh.) Hampe., *Umbilicaria polyphylla* (L.) Baumg., *Melanelixia subaurifera* (Nyl.) Essl., *Melanelia fuliginosa* (Fr. ex Duby) Essl.	Antitumor, antioxidant, antibacterial, antifungal, antidiabetic, anticancer, anti-inflammatory	[48, 51, 67, 72, 77, 78, 79, 80]
Lobaric acid	*Stereocaulon alpinum* Laur., *Heterodermia* sp.	Antibacterial, antifungal, anticancer, antineurodegenerative	[51, 81, 82, 83, 84]
Methyl evernate	*Ramalina fraxinea* (L.) Ach., *Ramalina fastigiata* (Pers.) Ach.	Antioxidant, antimicrobial, anticancer	[85]
Methyl β-orcinolcarboxylate	*Cladonia* sp.	Antidiabetic	[51, 86]
Methylorsellinate	*Cladonia* sp.	Antidiabetic	[86]
Norstictic acid	*Hypogymnia physodes* (L.) Nyl.	Antimicrobial, antioxidant, anticancer	[51, 78, 87, 88]
Olivetoric acid	*Pseudevernia furfuracea* (L.) Zopf., *Rhizoplaca melanophthalma* (DC.) Leuckert.	Antineurodegenerative, anti-inflammatory	[48, 51, 89]
Obtusatic acid	*Ramalina fraxinea* (L.) Ach., *Ramalina fastigiata* (Pers.) Ach.	Antioxidant, antimicrobial, anticancer	[76]
O-methyl anziaic acid	*Melanelixia subaurifera* (Nyl.) Essl., *Melanelia fuliginosa* (Fr. ex Duby) Essl.	Antioxidant, antimicrobial, anticancer	[76]
Psoromic acid	*Pseudevernia furfuracea* (L.) Zopf., *Rhizoplaca melanophthalma* (DC.) Leuckert.	Antineurodegenerative	[89]
Perlatolic acid	*Cladonia portentosa* (Dufour) Coem.	Antineurodegenerative, anti-inflammatory	[48, 50, 51]
Physodic acid	*Pseudevernia furfuracea* (L.) Zopf, *Hypogymnia physodes* (L.) Nyl., *Parmelia caperata* (L.) Ach., *Physcia aipolia* (Ehrh.) Hampe., *Evernia prunastri* (L.) Ach., *Umbilicaria polyphylla* (L.) Baumg., *Pseudevernia furfuracea* (L.) Zopf., *Rhizoplaca melanophthalma* (DC.) Leuckert., *Pseudoevernia furfuraceae* (L.) Zopf.	Antibacterial, antifungal, antioxidant, anticancer, antineurodegenerative, anti-inflammatory	[48, 50, 51, 53, 67, 72, 87]
Panarin	*Psoroma pallidum* Nyl., *Erioderma chilense* Mont.	Antioxidant, anticancer	[64, 90]
Parietin	*Teloschistes chrysophthalmus* (L.) Fr.	Antiviral, antimicrobial	[91, 92]
Protocetraric acid	*Hypogymnia physodes* (L.) Nyl., *Parmelia saxatilis* (L.) Ach., *Parmelia caperata* (L.) Ach.,	Antibacterial, antifungal, antioxidant, anticancer	[72, 78, 88, 93]

	Parmelia sulcata Taylor., *Physcia aipolia* (Ehrh.) Hampe., *Umbilicaria polyphylla* (L.) Baumg.		
Protolichesterinic acid	*Cetraria aculeata* (Schreber) Fr.	Antitumor, antibacterial, anticancer, antiinflammatory	[48, 51, 84, 94, 95]
Ramalin	*Ramalina terebrata* Hook.f. & Taylor	Antioxidant, antibacterial	[17]
Sekikaic acid	*Ramalina farinacea* (L.) Arc., *Ramalina roesleri* Nyl	Antioxidant, antibacterial, Antidiabetic, Antiviral	[51, 57, 65, 96]
Stictic acid	*Hypogymnia physodes* (L.) Nyl., *Parmelia caperata* (L.) Ach., *Physcia aipolia* (Ehrh.) Hampe., *Umbilicaria polyphylla* (L.) Baumg.	Antioxidant, antimicrobial, anticancer	[72, 97]
Salazinic acid	*Usnea longissima* Ach., *Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw.	Antitumor, antibacterial, antifungal, antioxidant, antidiabetic, antiviral	[10, 47, 51, 74, 93, 96]
Umbilicaric acid	*Umbilicaria nylanderiana* (Zahlbr.) H. Magn., *Umbilicaria aprina* Nyl., *Umbilicaria virginis* Schaer.	Antioxidant, antimicrobial	[98]
Usnic acid	*Usnea longissima* Ach., *Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw., *Cladonia arbuscula* (Wallr.) Rabenh., *Cladonia stellaris* (Opiz) Tonzar & Viezda., *Ramalina celastri* (Sprengel) Krog & Swinscow., *Heterodermia* sp.	Antiviral, antitumor, antioxidant, antibacterial, antifungal, antipyretic, analgetic, antiinflammatory, hepatotoxic, antiviral, antidiabetic	[46, 47 49, 51, 72, 74, 87, 88, 96, 99, 100, 101, 102, 103]
Variolaric acid	*Ochrolechia deceptionis* (Hue) Darb.	Antioxidant, anticancer	[59]
Vicanicin	*Psoroma pallidum* Nyl., *Psoroma pulchrum* Malme.	Antioxidant, anticancer	[59]
Vulpinic acid	*Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw., *Letharia vulpina* (L.) Hue.	Antimicrobial, anticancer	[72,103]
Zeorin	*Cladonia* sp., *Lecanora frustulosa* (Dicks.) Ach., *Parmeliopsis hyperopta* (Ach.) Arnold.	Antioxidant, antibacterial, antifungal, antidiabetic	[11, 86,104]
2,3-Dihydroxy-4-methoxy-6-pentylbenzoic acid	*Ramalina farinacea* (L.) Arc.	Antiviral	[65]
2,3-Dihydroxy-4-methoxy-6-propylbenzoic acid	*Ramalina farinacea* (L.) Arc.	Antiviral	[65]
3,4-Methylenedioxy-3□-methoxybibenzyl	*Ramalina farinacea* (L.) Arc.	Antiviral	[65]
4□.Omethylnorhomosekikaic acid	*Ramalina farinacea* (L.) Arc.	Antiviral	[65]

4□-O-methylnorsekikaic acid	*Ramalina farinacea* (L.) Arc.	Antiviral	[65]
2,4-Dihydroxy-6-propyl	*Ramalina roesleri* Nyl.	Antioxidant	[57]
1□-Chloropannarin	*Psoroma pallidum* Nyl., *Erioderma chilense* Mont.	Antioxidant	[64]
5-Hydroxysekikaic acid	*Ramalina farinacea* (L.) Arc.	Antiviral	[65]
2-Dehydro-5-oxysekikaic acid	*Ramalina farinacea* (L.) Arc.	Antiviral	[65]

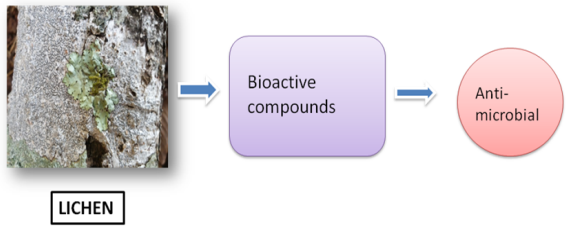

Figure 1 Lichen metabolites as potential antibiotic agent

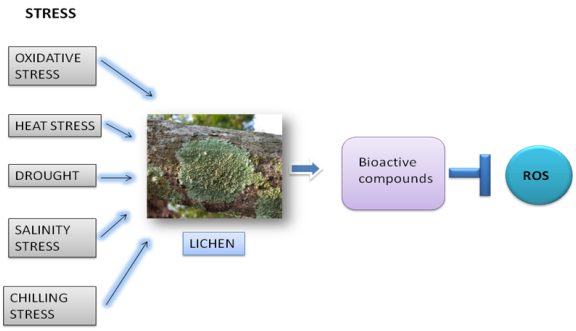

Figure 2: Potential antioxidant activity of lichen through ROS neutralization

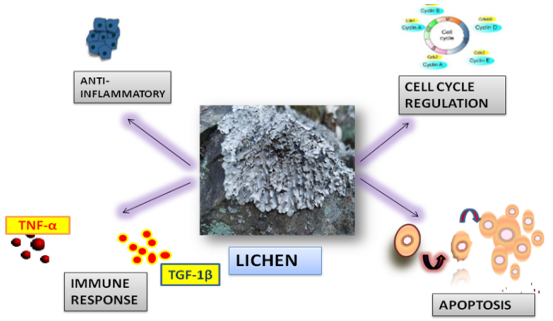

Figure 3. Potential anticancer activity of lichen metabolites

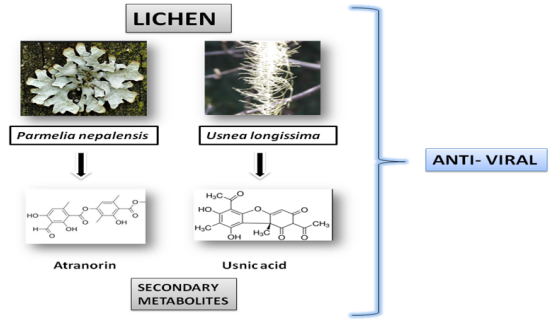

Figure 4 The potential bioactive compounds of lichen with antiviral properties

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RESEARCH ARTICLE

Cytotoxicity of Neem Extract on L929 Fibroblast Cell Line

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ABSTRACT

The aim of this study was to evaluate the cytotoxicity of endodontic intracanal medicament like ethanolic neem leaf extract and calcium hydroxide on L929 fibroblast cell line. Neem leaf extract and calcium hydroxide was used to evaluate the cytotoxicity. L929 cells were procured, placed in 25 cm^2 culture flasks and cultured in RPMI 1640 culture medium, with 10% fetal bovine serum, L–glutamine ,1% penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37°C in a humidified CO2 (5%) chamber and 95% air. The cells were detached using 0.25% EDTA Trypsin. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells (4, 9, and 14). The plates were placed on a shaker for 15 min and the absorbance was read on an enzyme linked immunosorbent assay (ELISA) reader at 570 nm. The percentage of cell viability was calculated as the relative absorbance of sample versus control wells as follows: % cell viability = (mean optical density of experimental well/mean optical density of control well) ×100. In this context, treatment of L929 fibroblasts with 10mg/mL of ethanolic neem leaf extract and calcium hydroxide resulted in 90% and 74.45% cell viability, respectively. Concentration played a significant role in the cytotoxicity profiles of endodontic intracanal medicament ethanolic neem leaf extract and calcium hydroxide. Calcium hydroxide became more toxic with exposure to cells when compared with ethanolic neem leaf extract medicament.

Keywords: NEEM extract, Cytotoxicity, MTT Assay, Intracanal medicament, Calcium hydroxide.

INTRODUCTION

Intracanal medicaments should be considered at the concentrations which are bactericidal and also the effect on the cell viability in the periapical tissues must be used at concentrations that are bactericidal while having minimal effects on cell viability in periapical tissues. Calcium hydroxide is the most commonly used medication for root canal disinfection. Because of its high pH, calcium hydroxide can lead to chronic inflammation and cell necrosis in vivo. There has been considerable discussion about finding alternate intracanal medications as an adjunct to irrigation and mechanical cleaning for the elimination of bacteria with minimum side effect and maximum biocompatibility. In the recent times an exponential growth in the field of herbal medicine has been observed and these drugs are gaining popularity both in developing and developed countries because of their natural origin and reduced side effects (1, 2).Many traditional medicines in use are derived from medicinal plants, minerals and organic matter and a number of medicinal plants, termed rasayana are used in the Traditional Indian health care systems have been in use for over 1000 years. Among the 21,000 plants listed by The World Health Organization (WHO) which are used for medicinal purposes around the world, 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is very aptly known as the botanical garden of the world (3, 4).

Azadirachta indica A. Juss., commonly known as neem and belongs to the Meliaceae family is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants(5, 6). Several pharmacological activities and medicinal utilities have been described, especially for leaf and stem bark. The leaf of this plant and its constituents have been demonstrated to exhibit immune modulatory, anti-inflammatory, anti-hyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties (7). In addition, leaves of *Azadirachta indica* has been reported to possess hepatoprotective (8), antifertility (9), antiulcer (Chattopadhyay et al., 2004), antimalarial (Udeinya et al., 2008) and anti-inflammatory (10) activities. The purpose of this laboratory study was to evaluate the cytotoxic effects of endodontic intracanal medicaments ethanolic neem leaf extract and calcium hydroxide on an established L929 cell line.

MATERIALS AND METHODS

Preparation of Neem Extract

Neem leaves were shade dried, powdered and stored in air-tight containers. They were soaked at room temperature for 24 hours, then filtered. Air dried Neem powder was repeatedly macerated with 500ml of 99% ethanol and filtered using whatman filter paper. The ethanol was evaporated and the extracts were concentrated using rotary flash evaporator and used in the assay (10, 11).

Extraction Methods

The final concentration was maintained as 1mg/ml by redissolving the neem extract in 10% dimethylsulfoxide for bioassay analysis and into 100μg/ml, 75μg/ml, 50μg/ml and 25μg/ml concentrations needed for the bioassay.

Maintenance of cell lines

L929 cells were procured, placed in 25 cm^2 culture flasks and cultured in RPMI 1640 culture medium, with 10% fetal bovine serum, L–glutamine, 1% penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C in a humidified CO_2 (5%) chamber and 95% air. The cells were detached using 0.25% EDTA Trypsin. Neutralization of the Trypsin was achieved using DMEM containing 10% FBS and PSGF, and cells were mechanically separated using a pipette. There were 96-well plastic culture plates filled with 200μl of medium containing in each well. The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air for 24 h to permit attachment of the cells to the plates.

Cell viability assay

For cell viability assay, on 96-well plates the cells were seeded at a concentration of 5x103 cells/well followed by addition of test samples various concentration prepared in cell culture media. MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase (12). The L929 cells were seeded at the density of (1 × 103 cells/ml) were plated on into well plates and treated with oil for 24 h. The cells were permitted to adhere for 24 hours, and the growth medium (MEM) removed using micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess FBS (11, 13). Cell culture medium (DMEM) was used as negative control for assessment of cell viability. 1ml of medium (without FBS) containing different dilution of drugs were added in respective wells; 200 µl of MTT (5 mg/ml in PBS) were added to each well, and the cells incubated for a further 6-7 hrs in 5% CO_2 incubator.

After removal of the medium, 1ml of DMSO was added to each well and the positive control (Allantoin (6mg/ml)) was tested. The effect of test sample (25-100µg/ml) on cell growth inhibition was assessed as percent cell viability, where vehicle-treated cells were taken as 100% viable. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells (4, 9, and 14). The plates were placed on a shaker for 15 min and the absorbance was read on an enzyme linked immunosorbent assay (ELISA) reader at 570 nm. From the values obtained, the percentage cytotoxicity (IC50 value) was calculated. Each experiment was carried out in triplicate and the half maximal inhibitory concentration (IC50) of the test samples as the percentage survival of the cells was calculated according to the formula provided below:
Percentage of viable cell concentration was calculated thus:
Viability (%) = (Mean test OD/Control OD) ×100

Statistical analysis

Results were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference test. P value is less than 0.05 and was considered as significant.

RESULTS

The calculation for the percentage of cell viability was done by the formula:
% cell viability = (mean optical density of experimental well/ mean optical density of control well) ×100.
In this cytotoxicity assay, treatment of L929 fibroblasts with 10mg/mL of ethanolic neem leaf extract medicament resulted in 90% cell viability and calcium hydroxide medicament resulted in 74.45% cell viability. Cells subjected to ethanolic neem leaf extract medicament significantly were more viable and calcium hydroxide was less viable (P0.05) (Table 1) assessed by comparison of the cell viability. Thus, the cytotoxicity of calcium hydroxide and neem extract in these concentrations was moderate and mild

DISCUSSION

In this study cytotoxicity of ethanolic neem leaf extract and calcium hydroxide were evaluated with comparison because calcium hydroxide has been used as an intracanal medicament. These intracanal medicaments were used clinically and they are in close contact with periapical tissue (15). The endodontic intracanal medicaments has particular concern regarding its toxic effects which results in irritation and damage to the periapical tissues and they also interrupts the wound healing. Cell culture in-vitro tests helps in controlling certain variables and experimental factors which is not possible in in-vivo tests. Usage tests and implantation in-vivo tests provides advantage over in-

vitro test like host, material complex interactions (16-19). But for the initial screening of a endodontic intracanal medicament before clinical use in-vitro tests are widely used.

The dental material cytotoxicity was checked in cultured mammalian cells through variety of in-vitro tests. The integrity of cell membranes are assessed by various vital dyes or by radio labeled chromium and these tests will also monitor the cell wall permeability(20, 21). The cell proliferating ability has been measured by replication assays measured by radio labeled nucleotide analogue incorporation or by immunoassay during DNA synthesis. Morphological studies assess the cell surface changes and cellular cytoskeleton changes. The energy for anabolic activity is provided by the cell which is measured by functional assays and also these assays assess the end products of such activities (22).

To measure the mitochondrial dehydrogenase activity, tetrazolium salt MTT assay was used in this present study. In living metabolically active cells, a dark blue formazan product is formed from a yellow substrate, which is done by a active mitochondria. The chemical nature of the material which is being tested should be considered before the selection of the tests to be performed (23). A permeability assay cannot be performed in a material which will not alter the cell membrane permeability in order to determine the cytotoxicity of the material. Some materials does not alter the cell membrane permeability but alter the intracellular enzyme activities like MTA which is hydrophilic and only alter the release of ionic components, in this case MTT assay is chosen. So MTT assay is done in this present study for cytotoxicity assay

For evaluating toxicity, Ethanolic Neem leaf extract and Calcium hydroxide were tested. This evaluation was performed according to the method of previous studies (8, 18, and 25). In this study, quantitative assessment with cell functional tests were accomplished. Periapical inflammation or flare up mostly result because of the apical debris extrusion and extrusion of irrigating solution or intracanal medicaments or because of instrumentation beyond working length (26). In order to reduce these inflammation and flare ups and to stimulate good periapical healing, the endodontic medicament used should be biocompatible and should have antimicrobial property (27). Comparison of neem extract and calcium hydroxide, showed ethanolic neem leaf extract to be less toxic than calcium hydroxide on L929 fibroblast cell line (28). Due to these biological properties, neem extract medicament is safe to be used as an intracanal medicament even if it extrudes into the periapical region it does not cause any toxic effect and tissue damage.

CONCLUSION

Concentration of the endodontic intracanal medicaments ethanolic neem leaf extract and calcium hydroxide plays an important role in the cytotoxicity of the medicament. With decrease in the concentration, it shows minimal or no cytotoxicity. Calcium hydroxide shows more toxic effect with exposure to cells when compared with neem extract. But extreme caution should be exercised in using these medicaments in clinical conditions. The biocompatibility of neem extract as an intracanal medicament should be assessed by many detailed clinical trials and in-vivo studies.

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Table 1: Values of Ethanolic Neem Leaf Extract medicament and Calcium Hydroxide medicament

S.No	Treatment	Conc (µg/ml)	Absorbance 570nm
1	L929 untreated cells	-	0.426±0.12
2	Neem Extract	10	0.401 ± 0.29
		20	0.368 ± 0.11
		40	0.347 ± 0.08
		80	0.284 ± 0.08
		100	0.208 ± 0.19
3	Calcium Hydroxide	10	0.324 ± 0.22
		20	0.269 ± 0.16
		40	0.213 ± 0.12
		80	0.189 ± 0.11
		100	0.176 ± 0.10

Figure 1: L929 Cells seeding and Test drug treatment

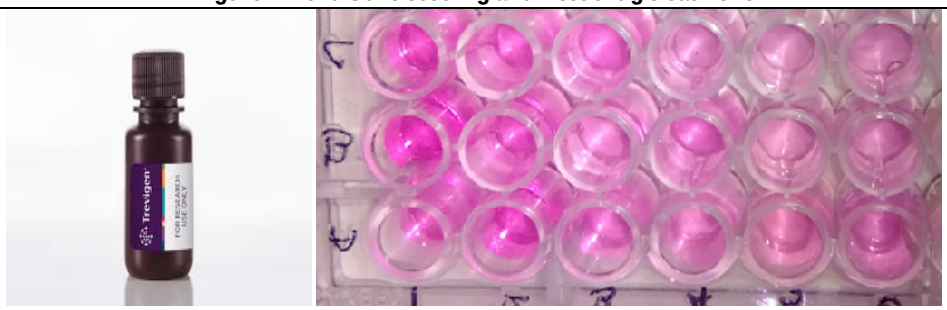

Figure 2: MTT chemical

Figure 3: Colour intensity read using microplate reader

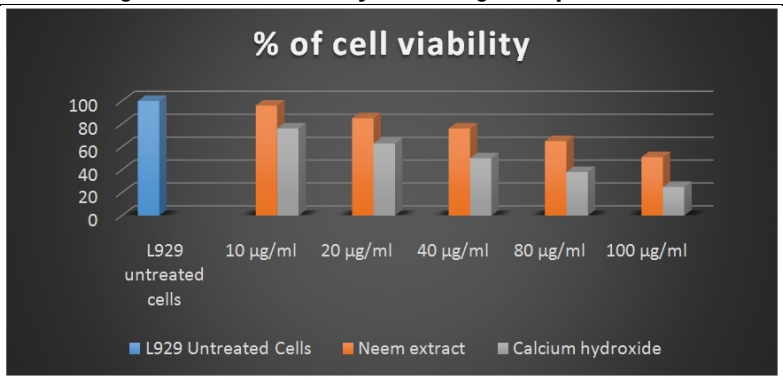

Chart 1: Percentage of cell viability

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RESEARCH ARTICLE

Impact of COVID-19 Outbreak and Total Lockdown on the Mental Health of Youth Population

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ABSTRACT

Covid-19 (Corona Virus Disease 2019) has significantly resulted in a large number of psychological consequences. Such widespread outbreaks are associated with adverse mental health consequences. The aim of this study is to explore the impact of Covid-19 outbreak on mental health of Youth population of Rajasthan state, India, to assist policy makers to develop actionable policies, and help clinical practitioners (e.g. social worker, psychiatrists and psychologist) provide services to affected population in time. Cross-sectional study design was used that included the demographic and sociological data for each participant and stress and frustration related information. Survey was conducted from 25 April to 05 May, 2020. The psychological impact of Covid-19 and lockdown was measured using the stress and frustration scale. Linear regression was used to calculate the univariate association between psychological impact scores and knowledge about Covid-19, precautionary measures variables, additional health information variables. The result of the study revealed that among 1210 respondents 944 had different level of stress with prevalence of 78 % and remaining 110 (22 %) were normal. Of all 944 respondents, 585(61.97%) reported mild psychological impact (score 11-25), 310(32.84%) reported moderate psychological impact (score 26-35) and 49(5.1%) reported severe psychological impact (score 36-50). During the lockdown period more than half of the respondent different level of stress viz: mild stress, moderate stress and severe stress. Our findings can be utilized to develop psychological interventions to improve mental health and psychological resilience during the Covid-19 epidemic.

Keywords: Corona Virus Disease; Pandemic; Cross-sectional study; Socio-demographic; Psychological impact; Stress.

INTRODUCTION

Covid-19 (Corona Virus Disease 2019) is a highly infectious disease with a long incubation period which caused by sars-Cov-2 (Severe Acute Respiratory Syndrome Corona virus 2). The disease since its first observed in China has now out spread to over 213 countries/territories, with report of local transmission occurring in more than 160 of these countries/territories. As per WHO (as of 27th May, 2020), there has been a total of 5,704,077 confirmed cases and 352.699 deaths due to COVID-19 globally [1]. WHO declared COVID-19 a pandemic on 11th March, 2020. While earlier the focus of spread was centered on China, it has now shifted to all over the World. WHO has suggested countries to take a whole-of-government, whole-of-society approach, built around a comprehensive strategy to prevent infection, save lives and minimize impact [2]. Widespread outbreaks of infectious disease, such as COVID-19, are associated with psychological distress and symptoms of mental illness [3,4,5].

The uncertainty and low predictability of Covid-19 not only threaten people's physical health, but also affect people's mental health, especially in terms of emotion and cognition, as many theories indicate. According to Behavioral Immune System (BIS) theory [6], people are likely to develop negative emotions (e.g. aversion, anxiety, etc.) and negative cognitive assessment for self protection [7,8]. Faced with potential disease threat, people tend to develop avoidant behaviors and obey social norms strictly [9]. According to stress theory [10] and perceived risk theory [11], public health emergencies trigger more negative emotion and affect cognitive assessment as well. However, long term negative emotion may reduce the immune function of people and destroy the balance of their normal psychological mechanisms. India is one of the youngest nations in the world and is expected to have a very favorable demographic profile in the near feature. There is also a need to monitor young people's mental health status over the long term and to study how prolonged school and college closures, strict social distancing measure, and the pandemic itself affect the wellbeing of our youth. Therefore it is essential to understand the potential psychological changes caused by Covid-19 in a timely manner

Objectives of the study

The objective of this study is to explore the impact of Covid-19 outbreak on mental health of Youth population, to assist policy makers to develop actionable policies, and help clinical practitioners (e.g. social worker, psychiatrists and psychologist) provide services to affected population in time

METHODOLOGY

Study Area

Rajasthan is the largest state of India administratively divided into 33 districts and 244 tehsils. As per Census 2011, the total population of the state is 6.68 crores which is approximately 5.6% of the country's total population [12]. The first case of the Covid-19 infection in Rajasthan, India was reported on 2nd March 2020 in Jaipur. The Rajasthan Health Department has confirmed a total of 7,376 cases, including 167 deaths and 4072 recoveries as of 26th May. 32 district in the state have confirmed cases of which, Jaipur is the worst-affected [13] (Figure-1)

Study Design

Cross-sectional study design was used that included the demographic and sociological data for each participant and stress and frustration related information. From 25 April to 05 May, 2020 survey was conducted in Rajasthan state India. The psychological impact of COVID-19 and lockdown was measured using the stress and frustration scale. Potential respondents were electronically invited by existing study respondents

Survey Development

Keeping in mind the purpose of the study and type of sample, questionnaire was considered most appropriate for gathering data. The questionnaire was prepared by the investigator in consultation with the subject matter

specialists. The questionnaire and scale were pre tested. On the basis of pre-testing, necessary modification was made. Pre-tested samples were excluded from the study. The content validity and reliability of the tool was obtained by experts in the field. Due to lockdown period respondent were completed the questionnaires through an online survey platform. The stress and frustration scale was divided into 0-10 (normal), 11-25 (mild psychological impact), 26-35 (moderate psychological impact), and 36-50 (severe psychological impact). The questionnaire responses were compared to determine the relationships between anxiety, stress and sleep. All the study participants were required to be able to provide informed consent to participate in the study. Data collection took place from 25 April to 05 May 2020

Sampling method

A snowball sampling strategy, focused on recruiting the youth (aged 15-27 year) living in Rajasthan state, India during Covid-19 pandemic, was used. A total of 1210 respondent were participated in this study.

Statistical Analysis

Descriptive statistics were used to analyze the data. The scores of the stress and frustration scale were expresses as mean and standard deviation. We used Chi- square to find out the association between socio demographic characteristics and scores of the scale. Linear regression was used to calculate the univariate association between psychological impact scores and knowledge about Covid-19, precautionary measures variables, additional health information variables. All tests were two-tailed, with a significant level of $p<0.05$. Statistical analysis was done by using SPSS statistic 21.0.

RESULTS

Development of COVID-19 Epidemic in India

Figure-2: shows the development trend of the COVID-19 epidemic in India from February 15 to May 25, 2020. Since Chine first announced the national epidemic data on 20 January 2020, the numbers of COVID-19 infection cases have continued to escalate Worldwide.

Psychological impact of Covid-19 outbreak on the respondents

The psychological impact of COVID-19 outbreak and lockdown measured using stress and frustration scale, revealed a sample mean score of 30.64 (SD=13.53). Among 1210 respondents 944 had different level of stress with prevalence of 78 % and remaining 110 (22 %) were normal. Of all 944 respondents, 585(61.97%) reported mild psychological impact (score 11-25), 310(32.84%) reported moderate psychological impact (score 26-35) and 49(5.1%) reported severe psychological impact (score 36-50) (Figure-3). Age wise psychological impact is shown in Figure-4. Wang et al. (2020) conducted a study to focus the general public living in mainland China during the epidemic of COVID-19. They also reported that 486 (51.48%) were considered to suffer from mild anxiety (score: 7–9); 358(37.92%) were considered to suffer from moderate anxiety (score: 10–14); and 100 (10.59%) were considered to suffer from severe and extremely severe anxiety (score: 15–42).

Socio demographic variables and psychological impact

The majority of respondents were female (69.59%). Half of the respondent (53.55%) were belonged to 17-20 year, 13.63% respondent were belonged to 15-17 year of age, 22.31% were 20-24 year of age and only 10.49% were 24-29 year of age. Majority (70.91%) of the subjects was the resident of urban area and 21.9% were from rural background. Only 19.4% subjects were from rural areas. With respect to religious category, result of the study indicates that most of the subject (74.05%) was Hindu and 7% were Muslims, 7.35% were Jain and only 3.55% were Christian. All the youth were educated. Among them 45.29% were graduate and 22.31% were post graduate. Working status of the subjects revealed that majority of the respondent (74.05%) were students and 25.94% were doing government/ private sector job or conducted their own business. Most of the respondents (72.31%) were from nuclear family and 27.69% were from joint family (Table-1).

Female respondent were significantly associated with higher scores of stress as compare to male gender. This observation corresponds to previously extensive epidemiological studies which found that women were at high risk of depression [14,15]. Working status of the respondents revealed that students were significantly associated with higher stress score as compared to those who were employed. As of March 14, 2020, all the schools and colleges have been suspended nationwide. The students of secondary, senior secondary and college student were very much worried about their studies. They were also in more stress because their annual/final board and university examination could not be done. Respondent having their own business also showed moderate level of stress and anxiety due to their financial loss during lockdown. All the religious people have mild to moderate level of anxiety and stress. As man is a social animal, social distancing for sometime due to any highly contagious and infectious disease including COVID-19 can lead to loneliness, anxiety and depression. All these psychological impact revealed in this study results.

Knowledge about Covid-19 and psychological impact

Knowledge of respondent regarding covid-19, revels that the most common route of transmission of Corona virus was through droplets (92.1%). Spread through contaminated objects (73.7%) and air born transmission (60.5%). Most of the respondent had heard that the number of Corona virus infected persons had increased (98.8%), the number of deaths due to Covid-19 had increased (97.8%), and the number of recovered individuals from Covid-19 infection had increased (93.3%). Internet (93.5%) was the common source of information about Covid-19. About 40% respondents were very much satisfied and 35% were fairly satisfied with the amount of health information available about Covid-19. Dissatisfaction with the amount of health information available about Covid-19 was significantly associated higher score of overall psychological impact of the outbreak of Covid-19 (B= 0.63, 95% CI: 0.11 to 1.14), and stress subscale score (B= 0.32, 95% CI: 0,02 to 0.62) (Table-2).

Precautionary Measures and Psychological Impact

The precautionary measures adopted by the respondents shown in Table-3. Nearly 80% of the respondent always or most of the time covering mouth when coughing and sneezing. More than half of the respondents (66.6%) were always washing hands after touching contaminated objects and always wearing mask regardless of the presence or absence of symptoms (59.8%). More than half of the respondents (59.8%) were always or most of the time washing hand with soap immediately after coughing, sneezing or rubbing nose. Avoiding the sharing of utensils during meal was significantly associated with the lower scores in over all psychological impact (B= -0.29, , 95% CI: -0.50 to –0.09), and the stress score (B= -0.18, 95% CI: -0.31 to –0.06), anxiety (B= -0.36, , 95% CI: -0.55 to –0.17). Similarly, washing hands immediately after coughing, sneezing, or rubbing the nose was significantly associated with lower scores in over all psychological impact (B= -0.47, 95% CI: -0.77 to –0.17), and stress (B= -0.31, 95% CI: -0.49 to –0.13), anxiety (B= -0.63, , 95% CI: -0.49 to –0.13), Washing hand with soap was significantly associated with lower scores in stress (B= -0.34, , 95% CI: -0.60 to –0.09), and anxiety (B= -0.54, 95% CI: -0.94 to –0.14). Infrequency of wearing masks regardless of the presence or absence of symptoms was significantly associated with higher scores in over all psychological impact (B= 0.52, 95% CI: 0.04 to 1.01). Washing hands after touching contaminated objects was significantly associated with lower scores in stress (B= -0.53, 95% CI: -0.96 to –0.10) (Table-3).

Additional Health Information Required and Psychological Impact

Additional Health Information Required by the respondent shown in Table-. Nearly all the respondent (86.66%) needs further health information about the Covid-19 infection. About 96.9% of respondent desired regular update for the latest information of Covid-19. Most of the respondent required information with respect to the route of transmission (96.9%), the availability and effectiveness of medicine/vaccines (96.8%), the number of infected cases and locations (94.1%), advice on prevention of the Covid-19 (93.7%), outbreaks in the local area (92.7%) and details on symptoms of Covid-19 Infection (91.9%).Additional information on the availability and effectiveness of medicines/vaccines (B= -0.63, 95% CI: -0.99 to –0.26), the number of infections and locations (B= -0.30, 95% CI: -0.57 to

–0.02), and the routes of transmission (B= -0.39, 95% CI: -0.77 to –0.02) were significantly associated with lower scores in anxiety scale (Table-4).

DISCUSSION

The finding of the study shows that psychological conditions significantly changed in Covid-19 Outbreak. With respect psychological responses of the youth population, 78% of respondents rated different level of stress from mild to severe. Severe depressive symptoms reported by 17.5% respondents, 25.6% reported moderate to severe anxiety symptoms and 7% reported moderate to severe stress levels. People showed more negative emotions (anxiety, depression and indignation) and less positive emotion (happiness, joy and hope) in Covid-19 pandemic, which was supported by the theory of BIS, i.e., people did generate more negative emotions for self-protection[16,17]. These results are consisted to previous studies as well, which found that public health emergencies (e.g. SARS) triggered a series of stress emotional response containing a higher level of anxiety and other negative emotions [18, 19].

In this study majority of respondent spent 20-24 hr per day at home. The main source of primary health information was internet and television. Nearly all the respondents (<90%) needed regular updates on the latest information on the route of transmission, numbers of Covid-19 cases and location, information on outbreak in the local area, details on symptoms and availability and effectiveness of medicines/vaccines. Majority of respondents were satisfied with the amount of health information available. Majority of respondents wore masks regardless of the presence or absence of symptoms and covered their mouth when coughing and sneezing as precaution strategies. More than half of the respondents washed their hands with soaps after touching contaminated objects. People show more concern for health and family, and less concern for leisure and friends.

As the Covid-19 pandemic continues to spread, our findings will provide vital guidance for the development of a psychological support strategy. Our findings have clinical and policy implications. Health authorities need to identify high- risk groups based on socio demographic information for early psychological interventions. Socio demographic data of the study revile that females had higher level of stress, anxiety and depression. This finding corresponds to previously extensive epidemiological studies which found that women were at higher risk of depression. [14]. Among the youth, psychological impact of the Covid-19 outbreak found higher in students (college students). As of March 14, 2020, all the schools and colleges have been suspended nationwide. The students of secondary, senior secondary and college student were very much worried about their studies. They were also in more stress because their annual/final board and university examination. The uncertainty and potential negative impact on academic progression could have an adverse effect on the mental health of the students. During the pandemic, education authorities need to develop online portal and web-based application to deliver lecture or other teaching activities [20]. As young people are more receptive towards smart phone applications, health authorities could consider providing online or smart phone based psycho-education and psychological interventions to reduce the risk of virus transmission by face-to-face therapy [21]. Online plate forms could also provide a support network for those people spending most of their time at home during the epidemic. Government and health authorities need to provide accurate health information during the epidemic to reduce the impact of rumors [22]. In our findings higher satisfaction with the health information received was associated with lower psychological impact of the outbreak and lower level of stress, anxiety and depression.

The theory of BIS supported that, people behave in a very reserve and conventional way when they feel threatened by disease [23]. Therefore, spending more time in home or staying at home with family and reducing entertainment activities seems to be safer way to prevent infection. It also showed that people became more conscious about their health and more likely to look for social support from their families rather than their friends, which advised that people interests and awareness were guided by the restricted travel policy and self-isolation regulations from the

health authorities and central and state government. The official guidance from the World health Organization (WHO) advise that healthy people should only wear masks if they are taking care of a person with suspected Covid-19 infection or if people are coughing and sneezing [24]. Present study found that wearing masks, regardless of the presence or absence of symptoms, was associated with lower level of anxiety and depression. Although the WHO emphasizes that masks are effective only when use in combination with frequent hand-cleaning with alcohol based hand rub or soap and water, wearing a mask regardless of the presence and absence of symptoms could offer potential psychological benefits by offering a sense of security.

This study has certain limitations. Due to limited available resources and time sensitivity of Covid-19 outbreak, snowball sampling was adopted. The sampling was not based on random sampling and the study participants did not reflect the actual pattern of the general population. Therefore findings may not represent the entire youth population. Due to ethical requirements on anonymity and confidentiality, we were not allowed to collect details and personal information from the respondents. Other variables affecting youth mental health with respect to Covid-19 outbreak should be examined.

CONCLUSION

This study find that mental health problem remains serious among the most of youth group during Covid-19 pandemic and lockdown. During the Covid-9 outbreak and lockdown period more than half of the respondent different level of stress viz: mild stress, moderate stress and severe stress. More than half of the respondents assessed their psychological impact as moderate to severe and about one third reported moderate to severe anxiety. Female gender and Students were associated with a greater psychological impact of lockdown and outbreak and higher levels of stress, anxiety and depression. From my point of view, there is a big effect on our mental health, primarily negative ones. This condition is definitely stressful especially because it has been slowly building up and going on for a long time. Moreover there is unpredictability how and when corona virus will be cured. This panic adds up to the stress. The anxiety, stress, financial strife, grief, general uncertainty of this time will definitely lead to behavioral health crises. . Using social media data may provide timely understanding of the impact of public health emergencies on the public's mental health during the epidemic period. Our findings can be utilized to develop psychological interventions to improve mental health and psychological resilience during the COVID-19 epidemic.

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Table-1: Association between socio demographic variables and level of stress among respondents

Particular	Normal Respondents		Respondent with different level of stress		Total Respondent		χ^2	p value
	N	%	N	%	N	%		
Age (years)								
15-17	55	33.33	110	66.66	165	13.63	24.9272	p < .05.
17-20	112	17.28	536	82.71	648	53.55		
20-24	73	27.03	197	72.96	270	22.31		
24-29	26	20.47	101	79.52	127	10.49		
Gender								
Male	170	46.19	198	53.8	368	30.41	180.7615	p < .05
Female	96	11.4	746	88.59	842	69.59		
Family back ground								
Rural	117	41.88	154	58.11	265	21.9	162.3538	p < .05
Urban	107	12.47	751	87.52	858	70.91		
Mixed	48	55.17	39	44.82	87	7.19		
Religion								
Hindu	150	16.74	746	83.25	896	74.05	67.2599	p < .05
Muslim	28	32.55	58	67.44	86	7.11		
Christian	20	46.51	23	53.48	43	3.55		
Jain	41	46.07	48	53.93	89	7.35		
other	27	28.12	69	71.875	96	7.93		
Education								
Primary	28	57.14	21	42.85	49	4.04	123.21561	p < .05
Middle	24	55.81	19	44.18	43	3.55		
Secondary	29	30.2	67	69.79	96	7.3		
Higher Secondary	37	24.67	113	75.33	150	12.4		
Graduation	56	10.21	492	89.78	548	45.29		
Post Graduation	72	26.67	198	73.33	270	22.31		
Doctorate	20	37.04	34	62.96	54	4.47		
Working Status								
Students	153	17.08	743	82.92	896	74.05	97.74	p < .05
Govt./ Private sector Job	33	61.11	21	38.88	54	4.45		
Own Business	15	14.7	87	85.29	102	8.43		

Other	65	41.14	93	58.86	158	13.06		
Family size								
Nuclear Family	137	15.65	738	84.34	875	72.31		
Joint Family	129	38.51	206	61.49	335	27.69	73.7512	p < .05

Table-2: Association between knowledge and concerns about the Covid-19 and the psychological impact of outbreak.

		Psychological mpact of Event			Stress		
Variables	**N(%)**	**R^2**	**AR^2**	**B (95% CI)**	**R^2**	**AR^2**	**B (95% CI)**
Knowledge of covid-19 Route of transmission							
Droplets							
Agree	1115(92.1)	0.002	0.001	0.21 (-0.07 to 0.49)	0.003	0.001	0.15 (-0.01 to 0.32)
Disagree	13(1.1)			0.48 (-0.25 to 1.21)			0.09 (-0.34 to 0.52)
Do not know	82(6.8)			Reference			Reference
Contact via contaminated objects							
Agree	892(73.7)	0.001	<0.001	0.04 (-0.15 to 0.22)	0.003	0.001	0.20 (-0.13 to 0.09)
Disagree	94(7.8)			-0.04 (-0.34 to 0.26)			0.16 (-0.34 to 0.02)
Do not know	224(18.5)			Reference			Reference
Air born							
Agree	732(60.5)	0.002	<0.001	0.11 (-0.07 to 0.29)	0.001	0.001	0.04 (-0.07 to 0.14)
Disagree	225(18.6)			0.17 (0.05 to 0.40)			0.002 (-0.13 to 0.13)
Do not know	253(20.9)			Reference			Reference
Have you heard that the number of positive Covid-19 cases has increased							
Yes	1195(98.8)	0.001	<0.001	0.40 (-0.24 to 1.03)	<0.001	0.001	0.10 (-0.27 to 0.47)
No	15(1.2)			Reference			Reference
Have you heard that the number of Covid-19 death has increased							
Yes	1183(97.8)	0.001	<0.001	0.21 (-0.27 to 0.69)	0.001	<0.001	0.18 (-0.10 to 0.46)
No	27(2.2)			Reference			Reference
Have you heard that the number of individuals that have recovered from Covid-19 infection has increased							
Yes	1129(93.3)	0.001	0.001	0.19 (-0.47 to 0.09)	0.007	0.006	0.24 (-0.40 to -0.07)
No	81(6.7)			Reference			Reference

The main source of Covid-19 information							
Internet	1131(93.5)	0.003	<0.001	0.46 (-1.46 to 0.54)	0.007	0.004	0.25 (-0.83 to 0.34)
Television	62(5.1)			0.22 (1.26 to 0.83)			0.07 (-0.68 to 0.54)
Radio	1(0.1)			0.83 (-1.81 to 3.47)			1.33 (-0.22 to 2.89)
Family members	10(0.8)			0.47 (-1.73 to 0.80)			0.27 (-1.01 to 0.48)
Other sources	6(0.5)			Reference			Reference
Satisfaction with the amount of health information available about Covid-19							
Very satisfied	485(40.1)	0.018	0.014	0.02 (0.34 to 0.37)	0.014	0.011	0.09 (-0.30 to 0.13)
Somewhat satisfied	423(35)			0.23 (-0.13 to 0.59)			0.03 (-0.19 to 0.24)
Not very satisfied	211(17.4)			0.39 (0.01 to 0.77)			0.09 (-0.14 to 0.31)
Not satisfied at all	40(3.3)			0.63 (0.11 to 1.14)			0.32 (0.02 to 0.62)
Do not know	51(4.2)			Reference			Reference

Table-3: Association between precautionary measure taken in lockdown and psychological impact Covid-19 outbreak

Variables	**n(%)**	**Psychological impact of Event**			**Stress**		
		R^2	**AR^2**	**B (95% CI)**	**R^2**	**AR^2**	**B (95% CI)**
Covering mouth when coughing and sneezing							
Always	694(57.4)	0.009	0.006	0.02 (-0.34 to 0.37)	0.003	<0.001	0.02 (-0.19 to 0.23)
Most of the time	282(23.3)			0.18 (0.19 to 0.55)			0.09 (-0.13 to 0.31)
Sometime	106(8.8)			0.40 (-0.02 to 0.82)			0.12 (0.13to 0.36)
Occasionally	77(6.3)			0.18 (-0.26 to 0.62)			0.03 (-0.29 to 0.23)
Never	51(4.2)			Reference			Reference
Avoiding sharing of utensils during meals							
Always	490(40.5)	0.041	0.037	0.20 (0.50 to -0.09)	0.017	0.013	0.18 (-0.31 to -0.06)
Most of the time	207(17.1)			0.17 (-0.07 to 0.40)			0.01 (0.13 to 0.16)
Sometime	162(13.4)			0.23 (-0.02 to 0.49)			0.02 (-0.17 to 0.14)
Occasionally	156(12.9)			0.36(0.10 to 0.62)			0.03 (-0.12 to 0.18)
Never	195(16.1)			Reference			Reference
Washing hands with soap and water							
Always	684(56.5)			0.42 (-0.85 to 0.01)			0.34 (-0.60 to 0.09)
Most of the time	266(22)			0.12 (0.56 to 0.33)			0.29 (-0.56 to -0.03)
Sometime	127(10.5)	0.029	0.026	0.07 (-0.40 to 0.54)	0.11	0.007	0.22 (-0.50 to 0.07)

Occasionally	100(8.3)			0.13 (-0.35 to 0.62)			0.17 (-0.46 to 0.12)
Never	33(2.7)			Reference			Reference
Washing hands immediately after coughing, rubbing nose, or sneezing							
Always	496(41)			0.47 (-0.77 to -0.17)			0.31 (-0.49 to -0.13)
Most of the time	227(`18.8)			0.003 (-0.32 to 0.32)			0.17 (-0.36 to 0.02)
Sometime	227(`18.8)	0.042	0.039	0.02 (-0.30 to 0.34)	0.02	0.016	0.12(-0.32 to 0.07)
Occasionally	185(15.2)			0.14 (-0.19 to0.47)			0.08 (-0.28 to 0.12)
Never	75(6.2)			Reference			Reference
Wearing mask regardless of the presence or absence of symptoms							
Always	723(59.8)			0.19 (-0.59 to 0.21)			0.21 (-0.45 to 0.02)
Most of the time	263(21.7)			0.12 (-0.30 to 0.53)			0.09(-0.34 to 0.16)
Sometime	116(9.6)	0.026	0.023	0.16 (-0.29 to 0.61)	0.009	0.006	0.08 (-0.35 to 0.19)
Occasionally	69(5.7)			0.52 (0.04 to 1.01)			0.04 (-0.33 to 0.24)
Never	39(3.2)			Reference			Reference
Washing hands after touching contaminated objects							
Always	806(66.6)			0.11 (-0.69 to 0.47)			0.21 (-0.56 to 0.13)
Most of the time	283(23.4)			0.19 (-0.40 to 0.78)			0.15 (-0.49 to 0.21)
Sometime	66(5.4)	0.018	0.014	0.40 (-0.25 to 1.04)	0.007	0.003	0.01 (-0.39 to 0.38)
Occasionally	37(3.1)			0.31 (-0.39 to 1.00)			0.07 (-0.48 to 0.34)
Never	18(1.5)			Reference			Reference
Feeling that to much of unnecessary worry has been made about the Covid-19 outbreak							
Always	156(12.9)			0.47 (-0.69 to -0.25)			0.08(-0.21 to 0.05)
Most of the time	108(8.9)			0.19 (-0.44 to 0.07)			0.05 (0.20 to 0.11)
Sometime	242(20)	0.019	0.016	0.03(-0.21 to 0.16)	0.002	0.001	0.01 (-0.12 to 0.10)
Occasionally	166(13.7)			0.13(-0.09 to 0.34)			0.03 (-0.10 to 0.16_
Never	538(44.5)			Reference			Reference

Tables-4: Association between additional health information required by participants, and the psychological impact of the Covid-19 outbreak.

		Impact of Event			Stress		
Variables	**n(%)**	**R^2**	**AR^2**	**B (95% CI)**	**R^2**	**AR^2**	**B (95% CI)**
Need for further health information about the Covid-19 infection							
Yes	1048(86.6)			0.36 (0.15 to 0.57)			0.12 (0.00 to 0.24)
No	162(13.4)	0.01	0.009	Reference	0.003	0.002	Reference
Need for details on symptoms of the Covid-19 infection							
Yes	1108(91.6)			0.34 (0.09 to 0.59)			0.02 (-0.17 to 0.13)

No	102(8.4)	0.006	0.005	Reference	<0.001	0.001	Reference
Need for advice on prevention of the Covid-19 infection							
Yes	1134(93.7)			0.52 (0.23 to 0.81)			0.11 (-0.06 to 0.28)
No	76(6.3)	0.01	0.009	Reference	0.001	0.001	Reference
Need for advice on treatment of the Covid-19 infection							
Yes	1000(82.6)			0.19 (0.006 to 0.38)			0.03 (-0.08 to 0.14)
No	210(17.4)	0.003	0.003	Reference	<0.001	0.001	Reference
Need for regular updates for latest information about the Covid-19 infection							
Yes	1173(96.9)			0.03 (-0.44 to 0.38)			0.11 (-0.35 to 0.13)
No	37(3.1)	<0.001	0.001	Reference	0.001	<0.001	Reference
Need for the latest updates for outbreaks of the Covid-19 infection in the local area							
Yes	1122(92.7)			0.06 (-0.21 to 0.33)			0.01 (-0.15 to 0.17)
No	88(7.3)	<0.001	0.001	Reference	<0.001	0.001	Reference
Need for information on the availability and effectiveness of medicines/vaccines for the Covid-19 infection							
Yes	1171(96.8)			0.19 (-0.21 to 0.59)			0.16 (-0.40 to 0.07)
No	39(3.2)	0.001	<0.001	Reference	0.002	0.001	Reference
Need for travel advice for the Covid-19 epidemic							
Yes	1160(95.9)			0.07 (-0.28 to 0.14)			0.07 (-0.29 to 0.42)
No	50(4.1)	<0.001	0.001	Reference	<0.001	<0.001	Reference
Need for updates on how other countries handle the Covid-19 outbreak							
Yes	1144(94.5)			0.25 (-0.06 to 0.56)			0.008 (-0.19 to 0.18)
No	66(5.5)	0.002	0.001	Reference	<0.001	0.001	Reference

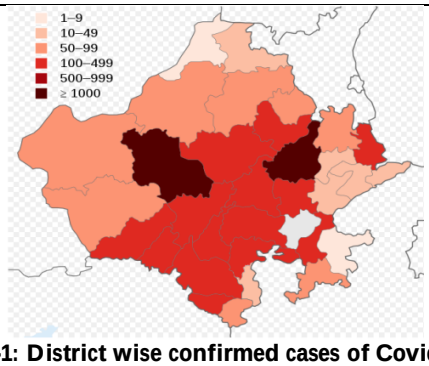

Figure-1: District wise confirmed cases of Covid-19 in the state Rajasthan

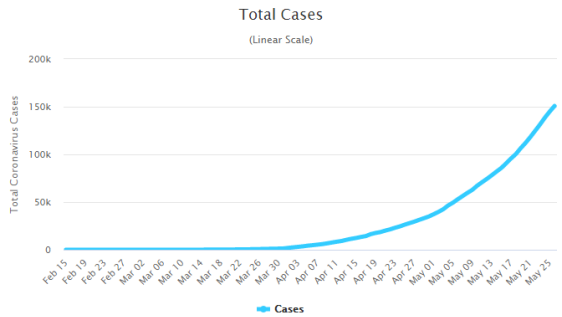

Figure-2: Corona virus situation in India (cases per day). (Source: MoHFW, 2020)

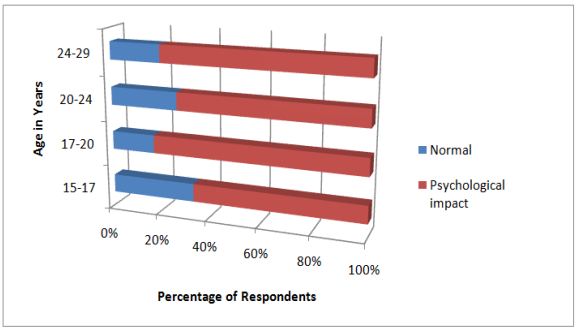

Figure-3: Psychological impact of Covid-19 outbreak on the respondents

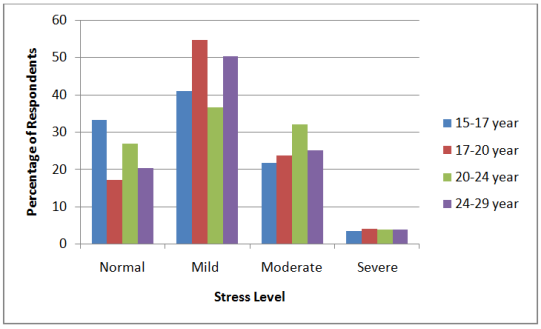

Figure-4: Stress level in different age group of Respondents