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Editorial

Greetings on the New Year!!

As Scientia (ISSN: 0976-8289) enters the 11th year of publication, we are delighted to present this issue with two review papers and 15 research papers touching all fields of science. This issue opens with an interesting review on "Zika virus" current epidemic causing devastating effects in many continents. The article describes its biology, etiology, potential association with microcephaly and Guillain–Barré syndrome and how it becomes a threat to mankind. Another review article focuses on Li-Fi (Light Fidelity) as an emerging communication technology. The research paper contributed by Dr.Kishore kumar on vascular flora of Anamudi Shola National Park, Munnar, Idukki district helps the budding researches and environmentalists as a chek list. This may motivate the future researches in the field of plant science to contribute in that area. The efficacy of titanium dioxide nanoparticles in Photo-decolorization was experimentally proved by a group of researchers in this volume. Two papers focused on Cladoceran embryogenesis and influence on light. Screening of mutation in BRCA genes and prediction of cancer susceptibility and genetic risk assessment was studied by another author. Biodiversity forms the key role in two studies. An in vitro study on the efficacy of commercially available mouthwashes on oral bacterial load in healthy individuals was attempted. Studies on the host preference and oviposition decisions in the pulse beetle, *Callosobruchus chinenesis* and Bioefficacy of selected botanicals in the management of rice weevil, Sitophilus Oryzae were discussed. Cytotoxic and Genotoxic effects of the Pesticide Ekalux (EC_{50}) on the root meristems of *Allium cepa*

and Imidacloprid effects on germination of seeds and associated physiological changes of cucumber (*Cucumis sativus* L.) were demonstrated through various experiments. The role of MSH2 gene product in relation to HNPCC disease was analysed using single nucleotide polymorphism (SNPs).

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We look forward for your critical analysis, suggestions and future contributions in the form of Minireview/Reviews/ research papers / short communications for inclusion in the next volume.

With warm regards,

Dr. Jayasree S.

(Chief Editor)

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The Zika Virus: A new Threat from mosquito

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Abstract

Zika virus disease (Zika) is a disease caused by the Zika virus, which is spread to people primarily through the bite of an infected *Aedes* species mosquito. Zika virus is a *flavivirus*, in the family *Flaviviridae*, distributed throughout much of Africa and Asia. The most common symptoms of Zika are fever, rash, joint pain, and conjunctivitis (red eyes). The illness is usually mild with symptoms lasting for several days to a week after being bitten by an infected mosquito. However, Zika virus infection during pregnancy can cause a serious birth defect called microcephaly, as well as other severe fetal brain defects. Once a person has been infected, he or she is likely to be protected from future infections. Zika virus is transmitted to people primarily through the bite of an infected *Aedes* species mosquito (*A. aegypti* and *A. albopictus*). No vaccine exists to prevent Zika virus disease (Zika).Treatment is focused on relieving the symptoms. Prevention and control relies on reducing the breeding of *Aedes* mosquitoes and minimizing contact between mosquito vectors and people by using barriers (such as repellents, insect screens), reducing water-filled habitats supporting mosquito larvae in and close to dwellings, and reducing the adult mosquito populations around at-risk communities. Zika is commanding attention because of an alarming connection between the virus and microcephaly, a neurological disorder that results in babies being born with abnormally small heads. It causes severe developmental issues and sometimes death.

Keywords: Zica virus, flavivirus, microcephaly, *Aedes aegypti*

Introduction

In the early part of the 21st century has seen an unparalleled number of emerging infectious disease events: West Nile virus across the Americas, severe acute respiratory syndrome in China and beyond, chikungunya, avian infl uenza, Middle East respiratory syndrome coronavirus, Ebola virus. The latest in this series of events is Zika virus (family *Flaviviridae*, genus *Flavivirus*), a mosquito-borne disease. Zika fever is caused by the Zika virus (ZIKV), an arthropod-borne virus (arbovirus). The Zika virus is a member of the *Flavivirus* genus in the family *Flaviviridae*. It is related to dengue, yellow fever, West Nile and Japanese encephalitis, viruses that are also members of the virus family Flaviviridae[1]. Zika virus was first isolated from a sentinel rhesus monkey placed in the Zika Forest near Lake Victoria, Uganda in April 1947[2]; a second isolation from the mosquito *Aedes africanus* followed at the same site in January 1948[3] .Epidemiological studies point to a widespread distribution of ZIKV in the northern half of the African continent, as well as in many countries in Southeast Asia, including Malaysia, India, the Philippines, Thailand, Vietnam, Indonesia, and Pakistan[4-7]. Many different *Aedes* species mosquitoes can account for the transmission of ZIKV, including *Aedes aegypti* [8,9], which at present is considered to be the main vector of the virus in South and Southeast Asia[9] . Despite this broad geographical distribution, human ZIKV infections remained sporadic and limited to small-scale epidemics for decades, until 2007, when a large epidemic was reported on Yap Island, a territory of the Federated States of Micronesia, with nearly 75% of the population being infected with the virus[10]. Moreover, an outbreak of a syndrome due to Zika fever has been reported in French Polynesia, in addition to several cases of ZIKV infection in New Caledonia, Easter Island, and the Cook Islands, indicating a rapid spread of the virus in the Pacific[11].

Serological studies done in the 1950s showed that humans carried antibodies against Zika virus, and the virus was isolated from humans in Nigeria in 1968. Subsequent serological studies revealed evidence of infection in other African countries, including Uganda, Tanzania, Egypt, Central African Republic, Sierra Leone, and Gabon, as well as Asia (India, Malaysia, Philippines, Thailand, Vietnam, and Indonesia). Phylogenetic studies suggest the virus emerged in east Africa in the early part of the 20th century, later spreading to southeast Asia[12]. In 2007 there was a small outbreak in Yap, Federated

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States of Micronesia, and in 2013 a larger outbreak in French Polynesia, with 28 000 cases recorded in the first 4 months. Since the first reports of Zika virus infection in Brazil in early 2015[13], its rapid and explosive spread has resulted in an estimated 1·5 million cases with 4 million predicted across the continent by the end of the year, and the declaration by WHO of a Public Health Emergency of International Concern.

Fig.1 Countries that have past or current evidence of Zika transmission (as of March 2016)

In October 2015, Colombia reported the first autochthonous transmission of Zika virus outside Brazil,and by March 3, 2016, a total of 51,473suspected cases of Zika virus had been reported in that country. By March 2016, the virus had spread to at least 33 countries and territories in the Americas[16] (Fig. 1).On January 15, 2016, the Center for Disease Control and Prevention (CDC) issued a travel alert advising pregnant women to consider postponing travel to the following countries and territories: Brazil, Colombia, El Salvador, French Guiana, Guatemala, Haiti, Honduras, Martinique, Mexico, Panama, Paraguay, Suriname, Venezuela and the Commonwealth of Puerto Rico, because of the growing evidence of a link between Zika and microcephaly. The only intervention available for Zika virus is mosquito control, which, for *Aedes* spp mosquitoes, is notoriously difficult to sustain. Growing resistance to insecticides is an important issue, and breeding site destruction and the prevention of bites might be better ways forward. Unlike Ebola virus, for which there were vaccines on the shelf awaiting clinical evaluation, for Zika virus the cupboard is bare although investigators are working hard to fill it[17](Table.1).

Table.1.Pathogens without Vaccine[17]

No.	Pathogen	Virus
1	Ebola haemorrhagic fever virus	Hepatitis E virus
2	Lassa haemorrhagic fever virus	Zika virus
3	Marburg haemorrhagic fever virus	Enterovirus 68
4	Middle East Respiratory Syndrome coronavirus	Coxsackievirus 16
5	Severe Acute Respiratory Syndrome coronavirus	Paratyphoid A (Salmonella enterica)
6	Crimean–Congo haemorrhagic fever virus	West Nile virus
7	Chikungunya virus	Rift Valley fever virus
8	Nipah virus	Plague (Yersinia pestis)

There is a possible link between Zika fever and microcephaly in newborn babies by mother-to-child transmission[14], as well as a stronger one with neurologic conditions in infected adults, including cases of Guillain–Barré syndrome[15]. As per a WHO report, Zika is now present in 23 countries and Brazil, the hardest-hit country, has reported around 3,530 cases of the devastating birth defect, called microcephaly in 2015, that are strongly suspected to be related to Zika. In 2014, the virus spread eastward across the Pacific to French Polynesia, then to Easter Island and in 2015, to South America, Central America and the Caribbean. It is now considered a pandemic.

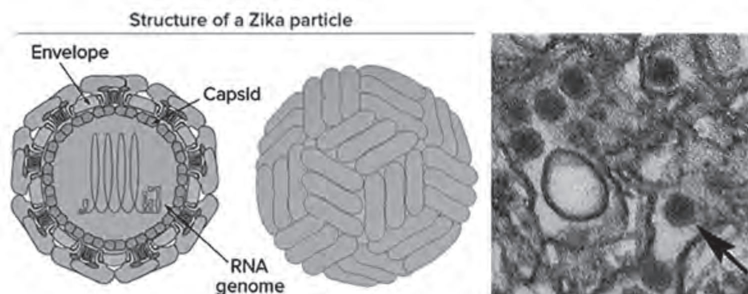

Fig.2. Image credit: left, modified from Zika virus: Virion, by ViralZone, Swiss Institute of Bioinformatics (CC BY-NC 4.0); right, Zika virus, by C. Goldsmith, CDC Public Health Image Library (public domain).

The virus

Zika virus (ZIKV) is a member of the virus family *Flaviviridae* and the genus *Flavivirus*. It is spread by daytime-active *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*. Its name comes from the Zika Forest of Uganda, where the virus was first isolated in 1947. Flaviviruses are tiny structures made up of protein, RNA (a molecule related to DNA), and a lipid membrane. Each viral particle consists of a single-stranded RNA genome tucked inside a protein shell called a capsid, surrounded by an external sphere of membrane known as the envelope . You can see Zika virus particles in the electron microcope image below right, where they appear as small, dark spheres. One particle is marked with an arrow.

A defining characteristic of viruses is that they cannot reproduce on their own. Instead, they must infect host cells and reprogram them to become virus-producing factories. Zika virus is no exception. It cannot replicate by itself, but can infect and replicate inside of the cells of several species, including humans, monkeys, and mosquitoes. Although we don't know which cell types Zika targets in the human body, studies with cultured cells (cells grown in a dish) show that Zika can infect a variety of immune cells found in human skin.

Virus classification

Group: Group IV ((+)ssRNA)

Family: *Flaviviridae*

Genus: *Flavivirus*

Species: *Zika virus*

Along with other viruses in the *Flaviviridae* family, Zika virus is enveloped and icosahedral with a non-segmented, single-stranded, positive sense RNA genome. It is most closely related to the *Spondweni virus* and is one of the two viruses in the *Spondweni virus* clade. Virus particles are 40 nm in diameter, with an outer envelope and a dense inner core. The Zika virus RNA is -10,617nucleotide long. The Zik a virus genome encodes for a polyprotein with three structural proteins, capsid, premembrane/membrane, and envelope (including the envelope1 54 - glycosylation motif previously associated with virulence), and seven nonstructural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 encodes for (Fig.3). Virions located on the surface of the cell membrane enter the host cells. The site of mRNA transcription is in the cell cytoplasm.

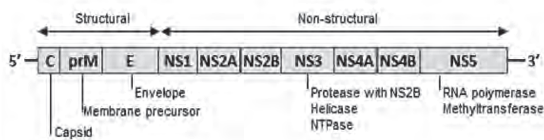

Fig. 3. Zika virus genome structure

Reproductive Cycle of a Zika virus in a Host Cell

Once Zika virus particles are in the human body, they must enter individual cells in order to replicate and make more viruses. Cell entry is possible because a Zika virus particle carries specific proteins on its outer envelope that interact with receptor proteins on human cells. When the viral proteins bind to cell receptors, they 'trick' the cells into taking up the viral particle. First, the virion attaches to the host cell membrane receptors via the envelope protein which induces virion endocytosis. Next, the virus membrane fuses with the endosomal membrane and the ssRNA genome

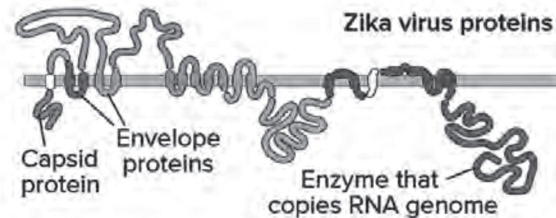

Fig.4. Image modified from Zika virus: Genome, by ViralZone, Swiss Institute of Bioinformatics (CC BY-NC 4.0).

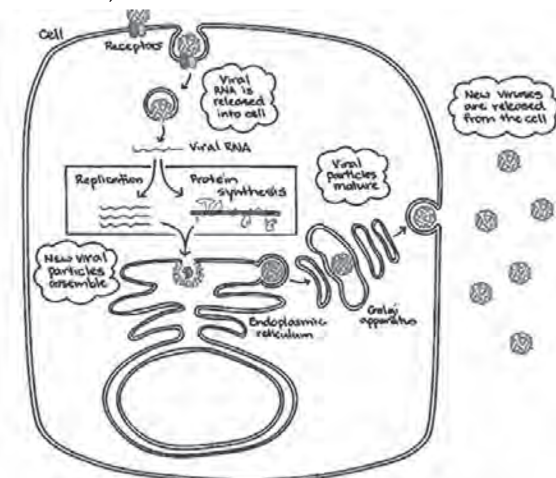

Fig.5. Zika virus life cycle

of the virus is released into the cytoplasm of the host cell. It is then translated into a polyprotein that is subsequently cleaved to form all structural and non-structural proteins. Replication then takes place at intracellular compartments known as cytoplasmic viral factories in the endoplasmic reticulum resulting in a dsRNA genome. The dsRNA genome is then transcribed resulting in additional ssRNA genomes.

Viral proteins and copies of the RNA genome assemble at the surface of the endoplasmic reticulum (ER), a membrane compartment that's part of the cell's export system. New viral particles bud off into the interior of the ER, taking a small patch of ER membrane along with them. This 'stolen' membrane will form the viral envelope. The particles then travel through another structure, the Golgi apparatus, where they undergo more processing before release at the cell surface. Assembly then occurs within the endoplasmic reiticulum and the new virions are transported to the Golgi apparatus and then excreted into the intracellular space where the new virions can infect new host cells. Released viral particles can infect other cells, continuing the infection cycle.

Transmission

Mosquito-borne Transmission

In Africa, Zika virus exists in a sylvatic transmission cycle involving nonhuman primates and forest-dwelling species of *Aedes* mosquitoes (Fig.6). In Asia, a sylvatic transmission cycle has not yet been identified. Several mosquito species, primarily belonging to the stegomyia and diceromyia subgenera of aedes, including *A. africanus, A. luteocephalus, A. furcifer,* and *A. taylori,* are likely enzootic vectors in Africa and Asia[18,19].

In urban and suburban environments, Zika virus is transmitted in a human–mosquito–human transmission cycle (Fig.6).Two species in the stegomyia subgenus of aedes , *A. aegypti* and, to a lesser extent, *A. Albopictus*[20] have been linked with nearly all known Zika virus outbreaks, although two other species, *A. hensil li* and *A. polynesiensis,* were thought to be vectors in the Yap[21] and French Polynesia[10] outbreaks, respectively. *A. aegypti* and *A. albopictus* are the only known aedes (stegomyia) species in the Americas. Despite the association of *A. aegypti* and *A. albopictus* with outbreaks, both were found to have unexpectedly low but similar vector competence (i.e., the intrinsic ability of a vector to biologically transmit a disease agent) for the Asian genotype Zika virus strain, as determined by a low proportion of infected mosquitoes with infectious saliva after ingestion of an infected blood meal [22]. However, *A. aegypti*is thought to have high vectorial capacity because it feeds primarily on humans, often bites multiple humans in a single blood meal, has an almost imperceptible bite, and lives in close association with human habitation[23].

The primary mode of Zika transmission is through mosquitoes in the genus *Aedes aegypti* and *Aedes albopictus* . When the insect bites a human, its snout probes around looking for a blood vessel. In doing so, it deposits virus particles in the victim's epidermis and dermis. To simulate infection in a laboratory, researchers inoculated a zika virus isolate, collected during the 2013 epidemic in French Polynesia, with three types of human skin

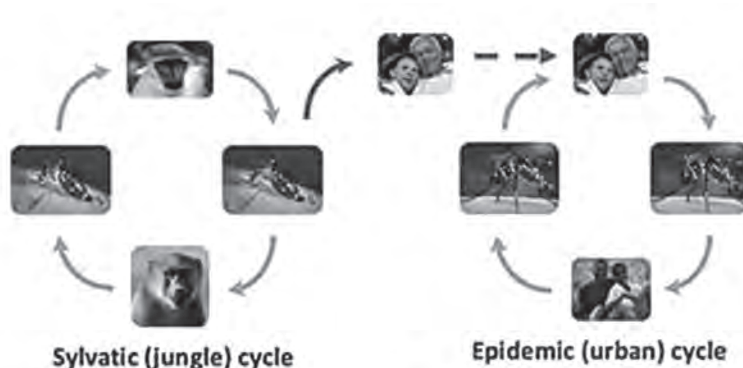

Fig.6. Zika Virus Transmission Cycle. A sylvatic transmission cycle between nonhuman primates and certain forest-dwelling species of *Aedes* mosquitoes. In suburban and urban settings-human–mosquito–human transmission cycle, involving *A. aegypti* mosquitoes.

cells, namely keratinocytes, found in the epidermis, and fibroblasts and dendritic cells, located in the dermis. The latter are immune system cells that playing a key role in the production of appropriate antibodies.When a mosquito eats a blood meal from a person infected with Zika, the virus can infect the cells of the mosquito. After the mosquito's infection has developed (usually after a period of about a week and a half), its saliva will contain viral particles. When it bites another human to obtain a blood meal, the viral particles can be transmitted to the human, who may contract Zika. Both *Aedes aegypti* and *Aedes albopictus* have been implicated in large outbreaks of Zika virus. *Ae. aegypti* is confined to tropical and sub-tropical regions, while *Ae. albopictus* can be found in tropical, sub-tropical and temperate regions. *Ae. albopictus* has spread from Asia and become established in areas of the South Pacific, Africa, Europe and the Americas in recent decades. The incubation period is typically between 2 and 7 days.

Non mosquito Transmission.

Substantial evidence now indicates that Zika virus can be transmitted from the mother to the fetus during pregnancy. Zika virus RNA has been identified in the amniotic fluid of mothers whose fetuses had cerebral abnormalities detected by ultrasonography, and viral antigen and RNA have been identified in the brain tissue and placentas of children who were born with microcephaly and died soon after birth, as well as in tissues from miscarriages. The frequency of and risk factors for transmission are unknown.Two cases of peripartum transmission of Zika virus have been reported among mother–infant pairs. Zika virus RNA was detected in both infants; one infant had a mild rash illness and thrombocytopenia, whereas the other was symptomatic.

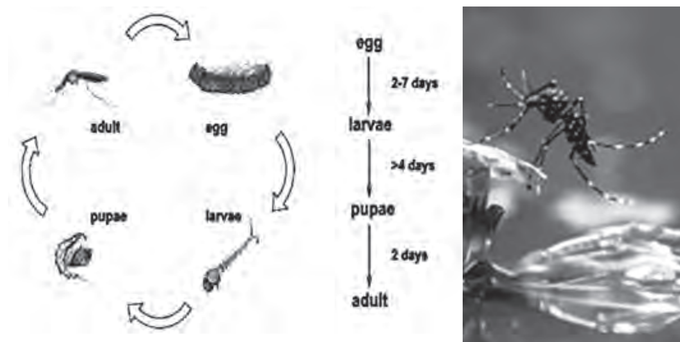

Fig.7 Life cycle of *Aedes aegypti*

There have also been a few documented cases of Zika transmission through sexual contact or through blood transfusions from a Zika-infected donor.

Life Cycle of *Aedes aegypti*

Aedes aegypti is a so-called holometabolous insect. This means that the insects goes through a complete metamorphosis with an egg, larvae, pupae, and adult stage. The adult life span can range from two weeks to a month depending on environmental conditions. The life cycle of *Aedes aegypti* can be completed within one-and-a-half to three weeks (Fig.6).

Egg

After taking a blood meal, female *Aedes aegypti* mosquitos produce on average 100 to 200 eggs per batch. The females can produce up to five batches of eggs during a lifetime. The number of eggs is dependent on the size of the bloodmeal. Eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man-made containers like barrels, drums, jars, pots, buckets, flower vases, plant saucers, tanks, discarded bottles, tins, tyres, water cooler, etc. and a lot more places where rain-water collects or is stored. The female *Aedes* aegypti lays her eggs separately unlike most species. Not all eggs are laid at once, but they can be spread out over hours or days, depending on the availability of suitable substrates. Eggs will most often be placed at varying distances above the water line. The female mosquito will not lay the entire clutch at a single site, but rather spread out the eggs over several sites.The eggs of *Aedes aegypti* are smooth, long, ovoid shaped, and roughly one millimeter long. When first laid, eggs appear white but within minutes turn a shiny black. In warm climates eggs may develop in as little as two days, whereas in cooler temperate climates, development can take up to a week. Laid eggs can survive for very long periods in a dry state, often for more than a year. However, they hatch immediately once submerged in water. This makes the control of the dengue virus mosquito very difficult.

Larvae

After hatching of the eggs, the larvae (Fig.8a) feed on organic particulate matter in the water, such

Fig.8a: *Aedes aegypti* larvae stage

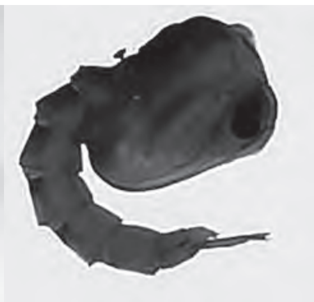

Fig.8b *Aedes aegypti* pupae

Fig.8c *Aedes aegypti* adult

as algae and other microscopic organisms. Most of the larval stage is spent at the water's surface, although they will swim to the bottom of the container if disturbed or when feeding. Larvae are often found around the home in puddles, tires, or within any object holding water. Larval development is temperature dependent. The larvae pass through four instars, spending a short amount of time in the first three, and up to three days in the fourth instar. Fourth instar larvae are approximately eight millimeters long. Males develop faster than females, so males generally pupate earlier. If temperatures are cool, ***Aedes aegypti*** can remain in the larval stage for months so long as the water supply is sufficient.

After the fourth instar, the larvae enters the pupal stage (fig.8b). Mosquito pupae are mobile and respond to stimuli. Pupae do not feed and take approximately two days to develop. Adults emerge by ingesting air to expand the abdomen thus splitting open the pupal case and emerge head first.

Signs and symptoms

Zika virus infection is characterized by low grade fever (less than 38.5°C) frequently accompanied by a macular or papular rash. Other common symptoms include muscle pain, non purulent conjunctivitis (55%), myalgia (48%), headache (45%), retro-orbital pain (39%), edema (19%), vomiting (10%) and arthritis (notably of the small joints of the hands and feet). Other symptoms that have been noted in association with acute infection include hematospermia, transient dull and metallic hearing, swelling of the hands and ankles, and subcutaneous bleeding. In a very small fraction of cases, Zika may be linked with neurological problems, such as Guillain-Barré syndrome. Guillain-Barré syndrome is an autoimmune condition that causes paralysis, which is usually temporary (lasting for weeks or a few months in most cases). A case control study in French Polynesia revealed a strong association (odds ratio, >34) between Guillain–Barré syndrome and previous Zika virus infection; the findings from electrophysiological studies were compatible with the acute motor axonal neuropathy subtype of Guillain–Barré syndrome[24].Meningoencephalitis[25] and acute myelitis[26]complicating Zika virus infection also have been reported.

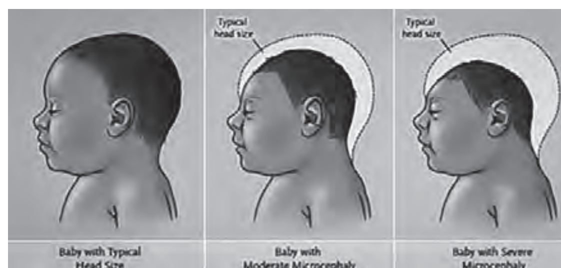

Fig.9 Infants with Moderate or Severe Microcephaly Associated with Maternal Zika Virus Infection, as Compared with a Typical Newborn.

Zika infection and the occurrence of fetal malformations, microcephaly (Fig.9) in newborns have also been reported. Microcephaly is a clinical finding of a small head size for gestational age and sex and is indicative of an underlying problem with the growth of the brain[27]. The lack of consistent and standardized case definitions has challenged the accurate monitoring of microcephaly during the current Zika virus outbreak[28]. Centers for Disease Control and Prevention (CDC) guidance has recommended that microcephaly be defined as an occipitofrontal circumference below the third percentile for gestational age and sex. Initial case reports from Brazil have suggested that some of the infants with microcephaly related to Zika virus infection have a phenotype consistent with fetal brain disruption (Fig. 8)[29]. The findings of Zika virus RNA in the amniotic fluid of fetuses with

microcephaly and in the brain tissue of fetuses and infants with microcephaly[30]as well as the high rates of microcephaly among infants born to mothers with proven antecedent acute Zika virus infection, provide strong evidence linking microcephaly to maternal Zika virus infection. The timing of the Zika virus and microcephaly epidemics in Brazil[31], and French Polynesia[32] indicate that the greatest risk of microcephaly is in the first trimester. In case reports of microcephaly, documented maternal Zika virus infec-tion most often occurred between 7 and 13 weeks of gestation, but in some cases it occurred as late as at 18 weeks of gestation[34].

Several methods can be used for diagnosis, such as viral nucleic acid detection, virus isolation and serological testing. Nucleic acid detection by reverse transcriptase-polymerase chain reaction targeting the non-structural protein 5 genomic region is the primary means of diagnosis, while virus isolation is largely for research purposes. Saliva or urine samples collected during the first 3 to 5 days after symptom onset, or serum collected in the first 1 to 3 days, are suitable for detection of Zika virus by these methods. Serological tests, including immunofluorescence assays and enzyme-linked immunosorbent assays may indicate the presence of anti-Zika virus IgM and IgG antibodies. Caution should be taken with serological results as IgM cross reactivity with other flaviviruses has been reported in both primary infected patients and those with a probable history of prior flavivirus infection.

The mainstays of the routine diagnosis of Zika virus infection are the detection of viral nucleic acid by RT-PCR and the detection of IgM antibodies by IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA). The detection of viral nucleic acid in serum provides a definitive diagnosis; however, in most instances viremia is transient, and diagnosis by RT-PCR has been most successful within 1 week after the onset of clinical illness[33]. RT-PCR and immunohistochemical testing have been useful in establishing Zika virus infection in tissues of fetal losses and full-term infants who died shortly after birth[34]. Brazilian ZIKV ($ZIKV^{BR}$) strain crosses the placenta and causes microcephaly by targeting cortical progenitor cells, inducing cell death by apoptosis and autophagy, and impairing neurodevelopment. The work reinforces the growing body of evidence linking the $ZIKV^{BR}$ outbreak to the alarming number of cases of congenital brain malformations. The study on model can be used to determine the efficiency of therapeutic approaches to counteracting the harmful impact of $ZIKV^{BR}$ in human neurodevelopment[35].

Treatment

As of 2016, no vaccine or preventative drug is available. Symptoms can be treated with rest, fluids, and paracetamol (acetaminophen), while aspirin and other nonsteroidal anti-inflammatory drugs should be used only when dengue has been ruled out to reduce the risk of bleeding. Bharat Biotech, a Hyderabad-based vaccines and bio-therapeutic manufacturer, on February 3,2016 claimed to have achieved a breakthrough in developing two vaccine to fight the dreaded mosquito-borne Zika virus. The inactivated vaccine, which consists of the disease-causing microbe which is killed with chemicals, radiation or heat, is a more stable and safer vaccine compared to the vaccines using live microbes. The other vaccine, known as Recombinant DNA vaccine, is based on the technology of using an attenuated virus or bacterium to introduce microbial DNA to the cells of the body.

Prevention and control

Mosquitoes and their breeding sites pose a significant risk factor for Zika virus infection. Prevention and control relies on reducing mosquitoes through source reduction (removal and modification of breeding sites) and reducing contact between mosquitoes and people. As with the other mosquito-borne flaviviruses, treatment for uncomplicated Zika virus infection focuses on symptoms. No Zika virus vaccine exists; thus, prevention and control measures center on avoiding mosquito bites, reducing sexual transmission, and controlling the mosquito vector.

Potentially effective methods of prevention that are focused on reducing infections among pregnant women include avoiding unnecessary travel to areas of ongoing Zika virus transmission, avoiding unprotected sexual contact with partners who are at risk for Zika virus infection, and using mosquito repellent, permethrin treatment for clothing, bed nets, window screens, and air conditioning. The most effective *A. aegypti* vector control relies on an integrated approach that involves elimination of *A. aegypti* mosquito breeding sites, application of larvicides, and application of insecticides to kill adult mosquitoes. However, each of these

approaches has substantial limitations. Communities are often mobilized to reduce *A. aegypti* breeding sites, but this strategy often fails, in part because of inconsistent participation among households and the presence of cryptic breeding sites in modern urban settings.Mosquito repellents should contain DEET (N, N-diethyl-3-methylbenzamide), IR3535 (3-[N-acetyl-N-butyl]-aminopropionic acid ethyl ester) or icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester). Product label instructions should be strictly followed. Special attention and help should be given to those who may not be able to protect themselves adequately, such as young children, the sick or elderly.

Non-essential travel to the affected countries in the Latin American region and the Caribbean should be deferred /cancelled. Pregnant women or women who are trying to become pregnant should defer/cancel their travel to the affected areas. All travellers to the affected countries/areas should strictly follow individual protective measures, especially during the day, to prevent mosquito bites (use of mosquito repellant cream, electronic mosquito repellants, use of bed nets, and dress that appropriately covers most of the body parts). Persons with comorbid conditions (diabetes, hypertension, chronic respiratory illness, immunity disorders, etc.) should seek advice from the nearest health facility, prior to travel to an affected country. Travellers who complain of fever within two weeks of return from an affected country should report to the nearest health facility. Pregnant women who have travelled to areas with Zika virus transmission should mention about their travel during ante-natal visits in order to be assessed and monitored appropriately.

Sexual transmission of Zika virus is possible. All people who have been infected with Zika virus and their sexual partners should practice safer sex, by using condoms correctly and consistently. Pregnant women's sex partners living in or returning from areas where local transmission of Zika virus occurs should practice safer sex, wearing condoms, or abstaining throughout the pregnancy.

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Li-Fi (Light Fidelity) as an emerging communication technology

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Abstract

At the time of using wireless internet at any place whether it is own or stealing from others, one has probably got frustrated because of the slow speed of internet when more devices are connected to a single router. Due to increasing of internet users exponentially, Radio spectrum is congested but the demand for wireless data double each year. Dr. Harald Hass has come up with a solution for those he calls "Data through illumination". LI-FI is a new epoch of high intensity light source of solid state design which bring clean lightning solutions to general and specialty lightning.LI-FI is now a part of the VLC as is implemented using white LED light bulbs. Data transmission takes place from this LED bulb by varying the current at extremely high speeds which undetectable by the human eye.

Keywords: LI-FI, WI-FI, Visible Light Communication, Radio Spectrum

Introduction

Harald Hass was coined the term LI-FI and promoted LI-FI in his 2011 TED Global talk by giving a demonstration on an LED light bulb to transmit a video with the speed more than 10 Mbps. German scientist succeeded in 2011 to creating an 800 Mbps (Megabits per second) capable wireless network by using nothing more than normal red, green, blue and white LED light bulbs, thus the idea has been around for a while and various other global teams are also exploring the possibilities. In simple terms, LI-FI can be thought of as it uses light instead of radio waves to transmit information. Instead if WI-FI modems or routers, LI-FI uses transceiver-fitted LED lamps that can be used as a light or for transmission of the data communication through internet. This technology uses a visible light communication spectrum and has not major ill effects as we know that the light is very much part of our life. Moreover in this spectrum 10,000 times more space is available and it also multiplies to 10,000 times more availability as light bulbs and street bulbs are available already.

Fig.1.LED Bulbs

Fig.2.Data communication medium

The figure shows the environment with the LI-FI technology where light bulbs are used as a data communication medium to PC, Laptop, Tablet and PDA.

Working Technology

LI-FI is implemented using white LED light bulbs which used for illumination by applying a constant current. However by fast variations of the current, the light output can be made to vary at extremely high speed. If the LED is on, it transmits a digital signal otherwise it transmits a digital 0.The LEDs can be switched on and off quickly to transmit the data that can't be detected by a human eye. There are also some enhancement could be made,

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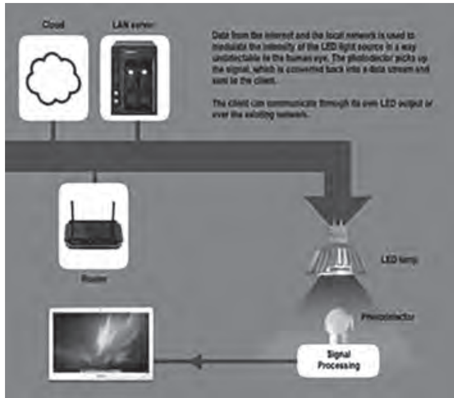

Fig.3.Working principle

like using an array of LEDs for parallel transmission, or using amalgamation of basic three colours i.e., red , green and blue LED's as different frequency with each having a different data channel. To further get a grasp of LI-FI consider an IR remote. It sends a single data stream with 10-20 kbps speed. Now if we replace the IR LED with a large LED array then can be capable of sending thousands of such streams at a very fast rate.The working procedure is very simple, if the light is on then transmit a digital 1's, if it's off transmit a 0. The LEDs can be switched on and off very quickly which gives nice opportunities for transmitting data.

Hence all we required is some LEDs and a controller that a code data into those LEDs. All one has to do is to vary the rate at which the LEDs flicker depending upon the data want to encode. Further enhancements can be made in this method, like using array of the LEDs for parallel data transmission or using mixtures of red, green and blue LEDs to alter the light's frequency with each frequency encoding a different data channel.

Data Transmission

As WI-FI hotspots and cloud computing are rapidly increasing reliable signal is bound to suffer. Speed and security are also major concern. They are vulnerable to hackers as it penetrates through walls easily.LI-FI is said to overcome this. This new technology is comparable to infrared remote controls which send data through an LED light bulb that varies in intensity faster than the human eye can see. In near future we can see data for laptops, smart phones and tablets transmitted through the light in a room.

Fig.4:Data transmission

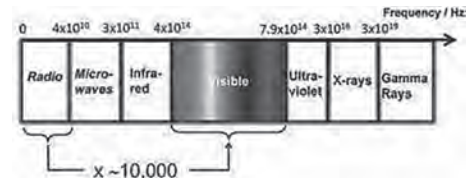

Fig.5:Visible Light

Why Visible Light Communication?

At first radio waves are expensive and less secure. Infrared, due to eye safety regulation can only base with low power. Gama rays cannot be used as they are dangerous. UV light is good for place without people, but otherwise dangerous for the human body. But visible rays are safe to use on larger bandwidth. VLC is a data communication medium, which uses visible light between 400 THz (780 nm) and 800THz (375 nm) as optical carrier for data transmission and illumination.

Brightness of LI-FI Source

The LI-FI source has a very high amount of light emitted per second in a unit solid angle from a uniform source (light intensity). A single source with only few millimeters in size can produce 2300 lumens of brilliant white light. In most cases, it will only need to use one light source per street light. It makes much simpler and less expensive.

What Makes A Fiber Optics Capable?

Fiber optic cables are made up of various parts

Fig.6. Fiber optics

depending on what cable we want. Generally fiber opticcables are wires that transmit data through an extremely thin layer of glass or plastic threads. These threads are actual fibers in a fiber optic cable. The relationship to LI-FI technology is in the regard that data travels through the fiber in the form of light which is then translated into 1's and 0's. One of the key

advantage to using light as a data transmitter is its massive bandwidth which is what makes fiber optics popular today.

Implementation

LI-FI is typically implemented using white LED light bulbs at the downlink transmitter. These devices are normally used for illumination only by applying a constant current. However, by fast and subtle variation of the current, the optical output can be made to vary at extremely high speed. This very property of optical current is used in LI-FI setup.Implementation of LI-FI given in the figure, and internet connection is connected to the lamp driver. A switch connected with lamp driver and LED lamp also connected with this lamp driver through fiber optics cable. Now a receiving device named photo detector is used for receiving signal and processing, this device is connected with PC or laptop's LAN port. On one end all the data on the

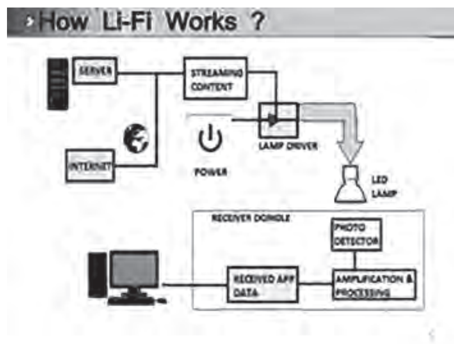

Fig.7. Implementation

internet will be streamed to a lamp driver. When the LED is switched on the microchip converts the digital data in the form of light.

The light sensitive device photo detector receives the signal and converts it back to original data. This method of using rapid pulse of light to transmit information wirelessly is technically referred as VLC.

Applications

There is a wide necessity for data transfer and by the end of the day every field involves the use of technologies. LI-FI proved its excellence in the following fields:

Spectrum Relief

Excess capacity demands of cellular networks can be off –loaded to LI-FI networks where available. This is especially effective on the downlink where bottlenecks tend to occur.

Mobile Connectivity

Laptops, smart phones and other mobile devices can be interconnected directly using LI-FI.

Hazardous Environment

LI-FI provides a safe alternative to electromagnetic interference from radio frequency communication in environment such as mines and petrochemical plants.

Aviation

LI-FI can be used to reduce weight and cabling and add flexibility to seating layouts in aircraft passenger cabins where LED lights are already deployed. In flight entertainment systems (IFE) can be supported and integrated with passenger's own mobile devices.

Underwater Communication

Due to strong signal absorption in water, RF use is impractical. Acoustic waves have extremely low bandwidth and disturb marine life. LI-FI provides a solution for short range communications.

Reduction in Accident numbers

At traffic signals we use LI-FI in order to communicate with LED lights of cars. By this number of accidents can be reduced. This can be used for vehicle-to-vehicle and vehicle-to-roadside communication.

RF Avoidance

Some people claim they are hyper-sensitive to radio frequencies and are looking for an alternative. LI-FI is a good solution to this problem.

Location Based Services(LBS)

Highly accurate location specific information such as advertising and navigation that enables

recipient to receive appropriate information in a timely manner and location.

Advantages

Fill Green Information Technology

LI-FI never gives any side effects on any living things like radio waves and other communication waves which effects on the birds, humans etc.

Free Form Frequency Bandwidth Problem

LI-FI is a VLC medium, so it does not requires any kind of spectrumlicense i.e. we don't need to pay for communication and license.

Smart Power Plants

Power Plants need fast and data systems with interconnected to monitors things like grid integrity, demand and core temperature and WI-Fi couldn't work properly in these areas but LI-FI could work properly in sensitive areas.

Increases Communication Security

Light can't penetrate to the wall so in VLC, security is higher than any other communication technology.

Multi User Communication

LI=FI helps to share multiple things at a single instance which supports the broad casting of network.

Features

Capacity

With the advent of new technologies like 3G, 4G we are running out of radio spectrum as its capacity are drying up. Compare to this VLC spectrum has more spectrum space than radio spectrum as light boxes are already present and already installed.

Efficiency

There are 14 lacks cellular radio base stations with efficiency of each station is just 15%.In base stations most of the energy has been used for cooling system.LI-FI is highly efficient because LED consumes less energy.

Security

Radio waves can penetrate walls and hence can be intercepted and misused. Light waves do not infiltrate through walls hence they are secured.

Availability

We have to switch off mobile phones in aircrafts and petrol pump is also area where mobile phones are to be switched off. Light is present everywhere and data is present everywhere light is present.

Comparison Of WI-FI & LI-FI

Challenges

Apart from many advantages over WI-FI, LI-FI technology is facing some problems such as LI-FI requires line of sight. A major challenge is how the receiving data will transmit data back to transmitter. Other disadvantage is visible light cannot penetrate through brick walls as radio waves and is easily blocked by somebody simply walking in front of LED source.

Li-Fi / Wi-Fi comparison

Parameter	Li-Fi	Wi-Fi
Speed	***	***
Range	*	**
Data density	***	*
Security	***	**
Reliability	**	**
Power available	***	*
Transmit/receive power	***	**
Ecological impact	*	**
Device-to-device connectivity	***	***
Obstacle interference	***	*
Bill of materials	***	**
Market maturity	*	***

* low ** medium *** high

Fig.8. Li-Fi verses Wi-Fi

Conclusions

If LI-FI technology can be put into practical use every bulb can be used to transmit data and will lead toward the cleaner, greener, safer and brighter future. It simply solves issues such as shortage of radio frequency bandwidth and is aimed at creating new communication channel with a use of existing equipment. Currently the LI-WI concept is attracting a great deal of interest.

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Imidacloprid effects on germination of seeds and associated physiological changes of cucumber (*Cucumis sativus* L.)

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Abstract

Vegetables are susceptible to insect and disease attacks, so pesticides are widely used. To combat the pests, growers use synthetic organic pesticides, and some biorational pesticides. Imidacloprid is a long-term systemic insecticide that is currently recommended for use on cucumber to control pests. It is one of the neonicotinoid insecticides with systemic activity that acts on the growth, physiological and biochemical characters of crop plants. Hence, the aim of the present investigation is to assess the effect of imidacloprid on seed germination and vigor potential of *Cucumis sativus* L. and to determine their associated physiological changes. The present study revealed the inhibitory effects on percentage of seed germination and vigor potential. The correlation coefficient among seed vigor index-I and II, and dry seedling weight and seedling length were recorded as 0.958 and 0.780 respectively. With the increasing concentrations of imidacloprid the abnormalities like swellings, chlorosis, rottening of cotyledons, burning of radical tips, decrease in the length of radicles and hypocotyls were been observed. The result also suggested that imidacloprid is linked with the imbalance in the activity of enzymes during seed germination process. In view of these findings, there is a dire need for dissemination of information regarding pesticide handling and good agricultural practices among farmers.

Key words: Pesticides, Imidacloprid, Cucumis sativus L., Seed vigor index, Seed germination

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most widely grown vegetable crops during *kharif* and summer season in all parts of the country. It is grown for its tender fruits which are consumed either raw as salad, cooked as vegetable or as pickling cucumber in its immature stage. It is a rich source of vitamin B and C, carbohydrates, Ca and P[1]. Cucumber plants are liable to infestation by many phytophagous pests such as the aphids, *Aphis gossypii* (Glover.) and the whitefly, *Bemisia tabaci* (Genn.), which considered the most common and important insect pests of cucumber plants. In case of heavy infestation, these pests are causing serious damage to plants, leading to great reduction in the yield[2]. With the implementation of the Food Quality Protection Act likely to limit the applications of some organic chemical insecticides, scientists and growers are seeking alternative materials that are effective against the pests and safe to humans and the environment[3, 4].

Imidacloprid [1-(6-chloro-3- pyridyl) methyl-4,5-dihydro-N-nitro-1-H-imidazol-2-amine)] are pesticides recommended for use on cucumber to control pests. Imidacloprid is an extensively used insecticide for crop protection in the world wide from the last decade due to its low soil persistence and insecticidal activity at low application rate[5]. It is fastest growing in sales as insecticide globally because of its low selectivity for insects and apparent safety for humans[6, 7]. Imidacloprid is highly effective against a wide range of economically important sucking insects for ornamental crops grown in greenhouses[8]. Most research reports on imidacloprid have been on field-grown plants, including tomato and cucumber, and most have not reported phytotoxicity symptoms[9, 10]. Only two reports were

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found that indicated phytotoxicity by imidacloprid, a reduction in seed germination of sugar beets[11] and marginal leaf necrosis of cauliflower[12], but the conditions that predispose plants to phytotoxicity are not known.

In India, extensive use of pesticides and insecticides in agriculture in recent years developed considerable interest in the study of their toxic effects on crop plants. Non-target plant effects include a wide range of symptoms including vegetative growth changes, plant death, altered reproductive capability that can generally result in reduced fitness and detrimental economics or ecological impacts, altered susceptibility to diseases of either the target or non-target plant may also be one of the unintended effects of pesticide. Hence, the present investigations have been undertaken to assess the effect of imidacloprid on seed germination and vigor potential of cucumber and to determine their associated physiological changes.

Materials and Methods

The present investigations were carried out with seeds of *Cucumis sativus* L. collected from Agricultural Research Station, Anakkayam, Malappuram, Kerala and subjected in the laboratory for seed germination, vigor tests, enzymatic assays and associated physiological changes.

Seed germination

Seed germination of *Cucumis sativus* L. was tested as per ISTA[13], in laboratory conditions through blot paper method. One hundred seeds were grown in four replications (25 seeds each). Seeds were allowed to germinate on the top of germination paper and final count was taken on 8th day. A sample of 25 seedlings from each replication was taken for vigor test. The dry weight (mg) and length (cm) of germinated seedlings were recorded. The average dry seedling weight was calculated by dividing the total dry weight of seedlings with number of seedlings and average length of seedlings was calculated by dividing the total length of seedlings with number of seedlings. Germination percentage of each replication was worked out by using following formula:

Germination percentage =

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds placed for germination}} \times 100$$

Seed Vigor Index

Seed vigor index was calculated as per the formula given by Abdul-Baki and Anderson (1973):

Seed Vigor Index - I =

Seed germination percentage x dry seedling weight (mg)

Seed Vigor Index - II =

Seed germination percentage x seedling length (cm)

Enzymatic assays

Preparation of Sample

Seeds of *Cucumis sativus* L. were placed in treated soil with different concentrations of imidacloprid. The germinating seeds were collected at 24 h intervals for 5 days. For each concentration, duplicate of 50 seeds were taken and ground in a cooled mortar for 15 min with acid washed sand in 0.2 M acetate buffer for protease activity and 0.2 M phosphate buffer for amylase activity. The ground materials were centrifuged at 8000 rpm for 10 min at 4°C. The supernatant was then collected and used for further assays.

Determination of Amylase Activity

Amylase activity was measured following the method described by Bernfeld (1955)[14]. A reaction mixture containing 0.5 ml of 1% soluble starch solution prepared in 0.2 M acetate buffer and 0.5 ml of diluted enzyme solution was incubated at 50°C. After 10 min of incubation the reaction was terminated by adding 1.0 ml of DNS solution (1 g of DNS dissolved in 20 ml of 2 M NaOH, to which 30 g of sodium potassium tartarate and water were added to make it 100 ml). Reaction mixtures were boiled for 15 min and after cooling 18 ml water was added. Absorbance was measured at 540 nm. One unit of enzyme activity was defined as the amount of enzyme that releases 1 micromole of glucose as reducing sugar in 1 min under the assay conditions.

Determination of Protease Activity

Protease activity was determined by caseinolytic method described by Walter (1984)[15]. 1 ml of enzyme solution was added to 2 ml of casein solution with 0.65 ml of 0.2 M glycine NaOH buffer and incubated at 37°C for 20 min. Reaction was terminated by adding 0.2 ml 1 N HCl and unhydrolyzed casein was precipitated with 5 ml of 5% TCA

solution and incubated at room temperature for 30 min. Thereafter, clear solution was separated by centrifugation at 9780 xg for 10 min. The filtrate was taken and 2 ml of 0.5 N NaOH, 0.6 ml of phenol reagent was added. The optical density was measured at 660 nm.

Statistical Analysis

After 8 days, the cumulative percentage of germination was determined. At the end of experiment, shoot and root length were measured in millimeters using a ruler. Treatments were arranged as a factorial combination of treatments in a completely randomized design with four replications. Twenty five seeds per replication were used for the germination test. Data were analyzed using an analysis of variance to detect differences between mean parameters. If significant differences were found, the least significant difference was used to determine mean differences between treatments.

Results and Discussion

Analysis of variance indicated significant differences among the characters under study viz seed germination, seedling length, dry seedling weight, seed vigor index-I and II (Table 1) and enzymatic assays.

The correlation coefficient among the different characters was worked out which revealed significant positive association with seed germination, dry seedling weight, seedling length, seed vigor index-I and II. Seed vigor index-I and II exhibited significant positive correlation with seed germination (0.810 and 0.835), dry seedling weight (0.955 and 0.910) and seedling length (0.820 and 0.935) respectively. Moreover seed germination percentage also exhibited significant positive correlation with dry seedling weight (0.653) and seedling length (0.527). The correlation coefficient among seed vigor index-I and II, and dry seedling weight and seedling length were recorded as 0.958 and 0.780 respectively. With the increasing concentrations of imidacloprid the abnormalities like swellings, chlorosis, rottening of cotyledons, burning of radical tips, decrease in the length of radicles and hypocotyls were been observed (Fig.1 and 2).

Similar correlations of yield with various seed characters in cucumber were also reported in previous literatures[16-18]. Degradation and dissipation residues of imidacloprid happened because the initial deposits and residues at different intervals of imidacloprid are influenced by different factors: evaporation of the surface residue which is dependent on temperature condition, biological dilution which is dependent on the increase mass of seeds, chemical or biochemical decomposition, metabolism and photolysis. Great interest to note that, the same factors were studied by several investigators. Christensen (2004)[19] reported that the decline of pesticides may due to biological, chemical or physical processes, or if still in the field, due to dilution by growth of the crop. Plant growth is also responsible to a great extent for decreasing the pesticide residue concentrations due to growth dilution effects[20]. In addition, the rapid dissipation of originally applied pesticide is dependent on a variety of environmental factors such as sunlight and temperature[21]. However, high temperature is reported to the major factor in reducing the pesticides from plant surface[22].

Table 1.Correlations of *Cucumis sativus* L seed characters

Traits	Seed Germination (%)	Dry seedling weight (mg)	Seedling length (cm)	Seed vigor index-I	Seed vigor index-II
Seed Germination (%)	1.00	0.653**	0.527**	0.810**	0.835**
Dry seedling weight (mg)		1.00	0.780**	0.955**	0.910**
Seedling length (cm)			1.00	0.820**	0.935**
Seed vigor index-I				1.00	0.958**
Seed vigor index-II					1.00

**Significant at 1% level of significance

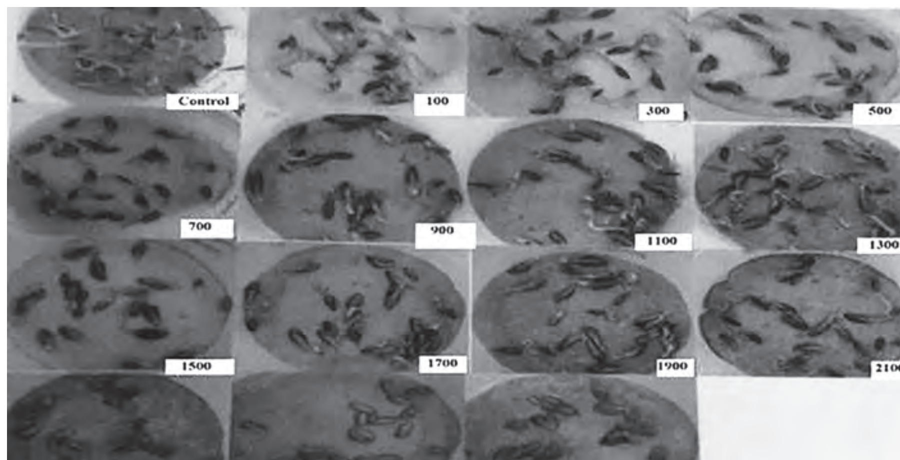

Fig. 1. Effect of different concentrations of Imidacloprid on germination seeds of *Cucumis sativus* L.

Fig.2. Effect of Imidacloprid on seedlings of *Cucumis sativus* L.

Biochemically, seed germination requires the solubilisation of stored polysaccharides; this is affected by denovo synthesis of amylase, which in turn is dependent on the embryonic growth and consequent release of giberrellic acid. Hence, amylase activity was investigated in the present study. It was observed that increased concentration of imidacloprid lead to a gradual decrease of activity of amylase. The activity of amylase was lowest in the highest concentration of imidacloprid viz., 2900ppm compared to 2500 and 2100ppm. Amylase is a hydrolytic enzyme which is responsible for degradation of storage polysaccharide pool during seed germination. In the present investigation, amylase activity was found to be lower in the seeds treated than control. Similar results were reported by Dalvi *et al.*, (1972)[23] in pesticide treated wheat and mung bean seeds.

Similarly, the amount of protease was also decreased in the 5th day. As concentrations increased, the activity of protease was found to be decreased. The minimum activity of protease was at highest concentration viz., 2900ppm. This is due to the inhibition of various proteolytic enzymes that degrade the storage proteins into polypeptides and free aminoacids. These degraded products remain in the storage tissue and transferred to the growing embryo axis during seed germination. The result obtained in the present investigation is linked with the imbalance in the activity of enzymes during seed germaination process.

In conclusion, the investigations of the present study indicate that imidacloprid can alter the plant physiological changes and its nutrition. Since growing conditions apparently affect sensitivity of plants to imidacloprid, additional studies need to be conducted to determine what growing conditions predispose plants to develop phytotoxicity symptoms. Nevertheless, pesticide residues in vegetables pose a significant health effect on human and animals. To provide adequate food for growing population, the usage of pesticide is necessary but dissemination of information regarding pesticide handling and good agricultural practices (GAP) among farmers is also a dire need.

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Biodiversity of aquatic insects of Thonnamakka canal of Karukutty Panchayath

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Abstract

Aquatic insects are extremely important in ecological system because of it is useful for bio-monitoring the aquatic ecosystems, Primary bio-indicators, Source of food and Nutrient recycling. Aquatic insects were collected from 5 different sampling sites of Thonnamakka canal of Karukutty panchayat, Ernakulam District from January 2015 to March 2015. Weekly collections were made from 5 sampling sites namely; Koramana, Attara, Edakkunnu, Thonnamakka, and Azhakom . Ecological parameters are also recorded. Insects are collected using pond net and collected insects are preserved in 70% ethanol and taxonomy status was assessed using the standard taxonomic keys. Insects collected from various sampling sites included in 13 family from 6 orders of phylum arthropoda namely, Coleoptera, Diptera, Ephemeroptera, Hemiptera , Odonata and Plecoptera. Most abundant group is hemiptera with 5 families. Order Hemiptera is highly specialized for aquatic mode of life and it was the most abundant order in this study. It was observed that Coleoptera specifically associated with the thick mat of aquatic vegetation and it was the second most recorded order. This may be due to the presence of wing known as elytra which may give extra protection to Coleoptera. Aquatic insects coming under the orders Plecoptera, Ephimeroptera, Coleoptera etc...could be good indicators for environmental pollution. They thrive more in polluted water. Since insects serve as the predators in the food web they are considered to be an important link in the food web. Larval and pupal forms of insects were also obtained from various sampling sites. Members from order decapoda were also identified during the study periodf insects were collected from Koramana and lowest is from Thonnamakka. As very little studies were conducted to identify the diversity of aquatic insects of Rivers of Kerala, the present study is very important as it may provide a benchmark data of the highly productive Rivers of Kerala.

Key words: aquatic insects, aquatic arthropods, bio-indicators, Thonnamakka canal

Introduction

The insects are the unique arthropods and have invaded almost all habitats. They occur commonly everywhere and differ widely in their form, structure and behaviour in relation to the mode of life they have adapted. Invertebrates like insects play a vital role in maintaining the health of all aquatic ecosystems by breaking down the organic matter and there by recycle both nutrients and energy. In general, these insects are considered as predators, which form an important component of the aquatic food chain. Aquatic insects are excellent indicators of both recent and long term environmental conditions. The immature stages of aquatic insects have short lifecycles, often several generations a year, and remain in the general area of propagation. Thus, when environmental changes occur, the species must endure the disturbances, adapt quickly, or die out and be replaced by more tolerant species. These changes often in as over abundance of a few tolerant species and the communities become destabilized or unbalanced.

Aquatic insects are very abundant and diverse group that inhabits a variety of environments. These organisms are an important component of aquatic [and sometimes terrestrial] food webs because they break down and process organic matter and provide food for invertebrates[1]. Despite their importance in aquatic ecosystems, very few insects spent their entire lives submerged in water. Most aquatic insects undergo an aquatic immature stage followed by a terrestrial adult. Even in cases where both the larvae and adult are aquatic, often the adult can exit in the water and or terrestrial. In rare cases, the larvae are terrestrial and the adult is aquatic. Aquatic insects are an important but often overlooked part of aquatic life. Ecologically, the insects perform a variety of function dependent on species. Aquatic insects are useful indicators of

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contaminations of the sediments and water that may have gone unnoticed by routine physico-chemical measurements[2].

Aquatic insects are the important links in nutrient recycling, they primarily process wood and leaf litter reaching the stream from the surrounding vegetation. Nutrients processed by aquatic insects of stream are further degraded into absorbable form by the fungal and bacterial action. Aquatic insects are also a primary source of food for fishes. In addition to this significant ecosystem function, aquatic insects are very good indicators of human impact on the freshwater ecosystem. Biological monitoring methods using aquatic insects have been developed and reliably tested in both temperate and tropical aquatic systems[3].

Aquatic insects are also useful indicators of contaminations of the sediments and water that may have gone unnoticed by routine physic-chemical measurements. Uptake of toxic substances, such as heavy metals and organochlorine compounds causes various kinds of deformities of larval and pupal Chironominidae[4]. Water chemistry and physical measurements alone are not sufficient to determine subtle shifts in aquatic populations. In a study on population dynamics of larval mosquito and succession in abundance of aquatic insects in the rice fields in Madurai, has shown a clear pattern in diversity of surface predators, bottom predators of mosquito immature, along with the increase in the mosquito larvae[5]. Biological monitoring methods using aquatic insects have been developed and reliably tested in both temperate and tropical aquatic system. Science of aquatic entomology embraces Odonates, Plecopterans, Ephemeropterans, Trichopterans, Dipterans and such others. They spent a part of (or) their whole life in aquatic system[6]. Biodiversity of aquatic insects of Karuvannur River, a part of Muriyad Wetland was undertaken and Coleopterans were the most abundant order[7]. Aquatic system offers multitude of ecological niche for aquatic, semi aquatic and terrestrial organisms. Almost all aquatic ecosystem of world are facing a serious threat of degradation and pollution due to human interferences.

Modern agriculture practices employing large quantity of chemicals and pesticides also contribute significantly in altering the normal ecological parameters of Aquatic system. Altered land use patterns in the uplands also directly affect the existence of umpteen numbers of flora and fauna depending on the Aquatic system

Materials and methods

Aquatic insects were collected from 5 different sampling sites of Thonnamakka canal which flows into Periyar River from January 2015 to March 2015. Weekly collections were made from the sampling sites namely; Koramana, Attara, Edakkunnu, Thonnamakka and Aazhakom. Ecological parameters like pH, water colour, temperature, aquatic vegetation were also simultaneously recorded from the sampling site. Pond net is employed to collect the insects. It was observed that coleopterans were specifically associated with the thick mat of aquatic vegetation; hence thorough search of this vegetation was carried out to collect the aquatic insects. The collected insects were preserved in 70% ethanol and taxonomy status was assessed using the standard taxonomic keys[8, 9]. Pupal and larval stages were also observed under microscope and photographed.

Results and Discussion

In the present study insects collected from various sampling sites included in 7 orders of phylum Athropoda; namely Coleoptera, Hemiptera, Odonata, Plecoptera, Ephemeroptera, Diptera and Orthoptera (Table 2). Members of order Decapoda was also recorded during the study period. Maximum insects collected during the study period were included in the order Hemiptera and then Coleoptera. Insects come under the family Velidae, Hydrometridae, Gerridae, Belostomidae and Nepidae of the order Hemiptera were identified. Insects come under the family curculionidae, Dytiscidae, Hydrophilidae, Coccinellidae were also identified. Larval and pupal stages of many insects like mosquito included in the order Odonata were also found. Members from the order Plecoptera, Odonata, Orthoptera and Ephemeroptera were also identified. Insects were collected more in early in the morning and evening. Samples obtained during day time were very low and more number of insects was collected from Koramana (Table 1).

Order Hemiptera is highly specialized for aquatic mode of life and it was the most abundant order in this study. It was observed that Coleoptera specifically associated with the thick mat of

Table1. Insects collected from various sampling sites

Sl. No.	Sampling sites	No. of insects collected
1	Edakkunnu	9
2	Attara	13
3	Koramana	16
4	Thonnamakka	8
5	Aazhakom	I2

Table2. Diversity of aquatic insects with their classification

Phylum	Class	Order	Family
Arthropoda	Insecta	Coleoptera	Coccinellidae
			Curculionidae
			Hydrophilidae
			Dytiscidae
		Diptera	Culicidae
		Ephemeroptera	Ephemeridae
		Hemiptera	Belostomidae
			Gerridae
			Hydrometridae
			Nepidae
			Velidae
		Odonata	Coenagrinidae
		Plecoptera	Eustenidae

aquatic vegetation and it was the second most recorded order. This may be due to the presence of wing known as elytra which may give extra protection to Coleoptera. Aquatic insects coming under the orders Plecoptera, Trichoptera, Ephimeroptera, Coleoptera etc. could be good indicators for environmental pollution. They thrive more in polluted water. Since insects serve as the predators in the food web they are considered to be an important link in the food web. Larval and pupal forms of insects were also obtained from various sampling sites. Members from order decapoda were also identified during the study period. As very little studies were conducted to identify the diversity of aquatic insects of Rivers of Kerala, the present study is very important as it may provide a benchmark data of the highly productive Rivers of Kerala.

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The embryogenesis of *Macrothrix triserialis* (Brady, 1886) (Cladocera: Macrothricidae) cultured in laboratory

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Abstract

The embryonic development of *Macrothrix triserialis* parthenogenetic female has been studied in the laboratory culture. The neonates were reared individually in petridishes and observed morphological changes of the embryo in the brood pouch throughout the development. The embryos were dissected out from the brood pouch at regular intervals and the mean size, morphological changes and duration of development were recorded. The culture was done at 26± 1^{0}C , pH 6.4± 0.3 and Chlorella density (1 x 106 cells ml-1). The results were compared with similar reports from Indian region.

Key words: Cladocera, *Macrothrix triserialis*, Embryogenesis.

Introduction

Macrothrix triserialis, is a littoral cladoceran (Anomopoda), most often found among submerged vegetation in freshwater bodies. This species was first described from Sri-Lanka[1]. In India, *M. triserialis* is first reported from Bihar[2] and later from Rajasthan[3, 4]. The first report of this species from Kerala is that of Michael and Sharma[4] based on the collections of Nayar, C.K.G from Irinjalakuda. Further report of this species is from Wynad[5] and from Thekkady[6]. The present study was done with specimens collected from a tropical wetland at Nellayi, Kerala.

Ramult was the first to experiment with Anomopodan embryos cultured *in vitro*[7]. Subsequently Fox[8], Green[9], Murugan and Venkataraman[10] , described the external appearance of developing embryo at certain intervals of time. Cladocerans, being important as live feed in aquaculture and hence, studies on biology may provide valuable information for their successful culture. The objective of the present study was to make a detailed examination of the embryogenesis of *M. triserialis* Brady.

Materials and methods

Macrothrix triserialis was collected with the help of a tow net made of bolting silk of 70µm mesh size. Taxonomic status was determined using monographs[4, 11, 12]. The collection was taken to laboratory in live condition and healthy egg bearing females were sorted out under a stereo zoom microscope Luxeo4Z. They were transferred into the culture medium kept under fluorescent light of uniform illumination (500 lux) in an aquarium (30 cm × 15 cm × 15 cm) containing live *Chlorel la* cells, nourished with finely powdered groundnut cake at a rate of 500 mg/litre[13]. A mild aeration was given throughout the culture period and maintained as a stock culture. Ten healthy egg bearing females were isolated from the stock culture with the help of a pipette and was inoculated into a beaker containing 200 ml. of same culture medium and observed until it released the neonates. The neonates produced were sorted out and individually reared in 40 petridishes (5 cm dia) at 26± 1^{0}C and pH 6.4± 0.3. They were watched to detect the entry of eggs into brood pouch which can be considered the initial starting moment of embryonic development. The morphological changes of the embryo in the brood pouch throughout the development were observed. At regular intervals, the embryos were dissected out from the brood pouch under a dissection microscope using micro-tungsten needles.The length of embryo was measured using

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calibrated micrometers and photos were taken using Sony digital camera. The end of embryogenesis was determined by the moult which occurred within a few minutes of the release of embryo from the brood pouch. Soon after moult, the embryo undergo considerable morphological changes and get transformed into a free swimming neonate.

Results and Discussion

The periodization of anomopod embryogenesis has been earlier reported by Green[9]. Following this classification, 3 well defined phases are recognized which include early, middle and late stage. The stages of embryonic development of *M. triserialis* in the present study are given below based on morphological changes at specific time intervals and divided into eight stages (Fig:1 a-h).

Stage I: Egg just released into brood pouch and starting of embryogenesis. This stage is recognized by the presence of oval-shaped egg with dark granular yolk. The egg starts divisions within a mean duration of 1.0 hr. Mean size: 0.176 mm (Fig. 1a)

Stage II: Embyo at 2.5 hrs. The embryo has two distinct areas. The outer area of the embryo appears transparent while the yolk granules are concentrated at the inner granular zone, cleavage is visible. Mean size: 0.180 mm (Fig. 1b)

Fig. 1 : *Macrothrix triserialis* – Stages of Embryonic development in a parthenogenetic female

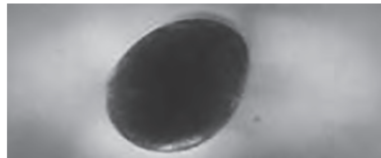

Fig. 1a Stage I (0.176 mm)

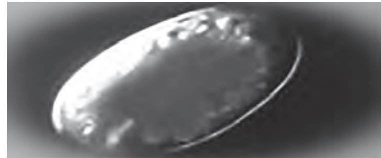

Fig. 1b Stage II (0.180 mm)

Fig. 1c Stage III (0.182 mm)

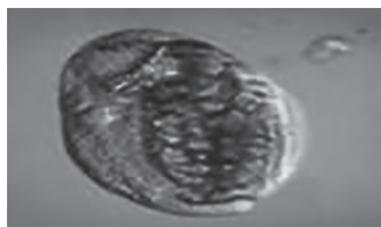

Fig 1d Stage IV (0.184 mm)

Fig. 1e Stage V (0.216 mm)

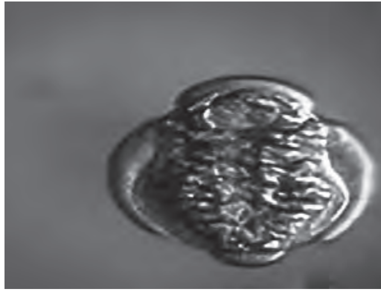

Fig. 1f Stage VI (0.220 mm)

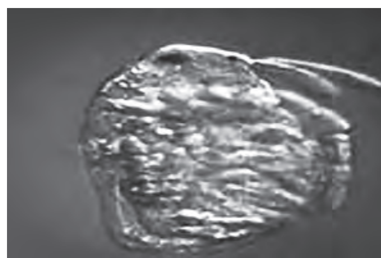

Fig 1g Stage VII (0.262 mm)

Fig 1h. Stage VIII (0.296mm)

Stage III: Embyo at 6.5 hrs. The embryo has elongated in anterio-posterior axis and shows cellular divisions. Mean size: 0.182 mm (Fig.1c).

Stage IV: Embyo at 9 hrs. The head lobe and antennary bud made their first appearance during this stage. Large yolk granules are very conspicuous in the centre and smaller granules are arranged in the periphery. Mean size: 0.183 mm (Fig.1d).

Stage V: Embyo at 12.0 hrs. During this stage the head lobe and the rudiment of antennae become more distinct, rudiments of appendages visible. The cellular divisions can be clearly seen and yolk gets fused in the centre. Mean size: 0. 214 mm (Fig.1e).

Stage VI: Embyo at 18.5 hrs. This stage can be recognized by the development of distinct rudiments of head, antennae, antennules, appendages and postabdomen. Mean size: 0.218 mm (Fig. 1f).

Stage VII: Embyo at 25.9 hrs. The eye become dark, ocellus appears. Setae appear on antennae, antennules and thoracic legs and postabdomen which become more distinct. Mean size: 0.266 mm (Fig.1g)

Stage VIII: Embyo at 29.7 hrs. The development of carapace, antennae, antennules, thoracic appendages, postabdomen and eyes completed. Setae of the antennae are very distinct. At this stage the embryos show morphological similarity to a neonate. Mean size: 0.298 mm (Fig. 1h).

The end of embryogenesis of *M. triserialis* is determined by the moult which occurs at 30.2±0.4 hrs and the neonates were released by the movement of postabdomen of the female. The present study as well as earlier studies made in *Daphnia magna*[9], in *Simocephalus acutirostratus*[14], in *Daphnia schodleri*[15], in *Moina micrura*[16], in *Daphnia carinata*[10] and in *Pleuroxus aduncus*[17] indicate that the stages of embryonic development follows a general pattern in Cladocera.

The total duration of embryonic development in *Daphnia hyalina*[19] is 70 hours at 22°C, 52 hours in *Daphnia magna*[9] and *Diaphanosoma brachyurum*[20] at 21 °C; where as the present study indicates that *M.triserialis* has a shorter duration of 29.7 hours at 26± 1°C. This shorter duration and faster embryonic development can be attributed to the comparatively higher water temperature. A relatively shorter duration of embryonic development is also reported in tropical species of *Scapholeberis kingi Sars*[18]. The rate of development is relatively faster in tropical species than their temperate counter parts which may be a survival strategy where rapid build of population become possibe before adverse environmental conditions set in[14].

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Studies on influence of light in ephippial production of *Simocephalus serrulatus* (Koch, 1841) (Cladocera: Daphinidae)

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Abstract

Considering the importance of *S.serrulatus* in larviculture and ornamental fish culture as live feed, a comprehensive study on the ephippial production is found to be essential for getting sufficient resting eggs to be used in mass culture techniques. For this the ephippial production was investigated by changing the duration of light exposure at 5 different photoperiods (12 L: 12D; 24 L: 0D; 0L: 24D; 08 L: 16D; 16L: 8D) in the laboratory for a period of 2 months. All the experiments were done at 26°C±2.0, pH 7.4±0.2 and D_{o2} 5.2±0.2 and observed the influence of light (1000 Lux) in the formation of ephippia. Duration of light exposure has been found to be an important factor in influencing ephippia formation. The ephippia were sorted out and kept in dark containers inside the refrigerator as an egg bank for future use. The results were discussed with earlier reports from Indian region.

Key words: *Simocephalus serrulatus,* Ephippial production, Photoperiod.

Introduction

Simocephalus serrulatus is a large cladoceran most often found among the littoral weeds and sediments of ponds. The first report of this species from India is by Michael and Sharma[1] from Meghalaya and Tamil Nadu; and later recorded from West Bengal and Southern Tamil Nadu by Venkataraman[2,3]. *S. serrulatus* has become a suitable food item for all fish larvae due to its high nutritive value, larger size, high availability and low expenditure in production. High levels of protein, free amino acids, fats and carbohydrates in cladocerans like *Daphnia carinata, D. longispina, D. magna* and *D.pulex,* have been reported[4] which are considered valuable live feeds. Cladocerans like *Moina* and *Daphnia* are important as live food organisms in freshwater larviculture and ornamental fish industry[5].

The reproduction in cladocerans can either be totally parthenogenetic (asexual) or may be intermixed with periods of gamogenetic (sexual) reproduction. Two female morphotypes are thus recognized in the populations which include the parthenogenetic individuals and ephippial forms. Although, parthenogenesis is the predominant mode of reproduction[6, 7]; they resort to sexual reproduction occasionally or seasonally. The production of males and ephippial females indicate the onset of sexual reproduction. The fertilized eggs which are enclosed inside the ephippia are called the resting eggs which undergo several cell divisions[8, 9] and further enter into a period of dormancy called 'diapause'. Nelson G. Hairston while studying the zooplankton egg banks observed that viable diapausing eggs can survive in aquatic sediments for decades or longer[10]. The development of an egg bank is one of the important methods to ensure a steady supply of desired organism for mass culture techniques; which also ensures the availability of this species throughout the year. The present study has made an attempt to identify the factors that influence the ephippia formation of this species. This will help to elucidate novel methods under laboratory conditions for stimulating the resting egg production in *S. serrulatus* and also to seek and explore the possibility of production of an egg bank.

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Materials and Methods

The first step in cladoceran culture is to ensure a reliable food source. Unicellular algae especially *Chlorella* has been used as food for freshwater cladocerans in laboratory culture and is known to support growth and reproduction[11]. The availability of unicellular algae is required for the successful culture of cladocerans. An algal stock culture was prepared and maintained in the laboratory to ensure a ready availability of unicellular algae. Water collected from a nearby pond containing *Chlorella* sps was filtered through bolting silk of 70μm mesh size and transferred into an aquarium (40 cm × 20 cm × 20 cm). This water was given a continuous aeration and kept under fluorescent light of 1000 Lux. A gold fish (*Carassius* sp.) was introduced into this medium so as to maintain the trophic system within the aquarium. The fish was fed with food pellets available in the market. The algal culture flourished and attained a dark green colour after 15 days. The algal density was estimated using a haemocytometer. The side walls of the aquarium were always kept clean to ensure sufficient penetration of light. For maintaining this culture for a long period the fish was fed daily, detritus was siphoned out from the bottom and the culture medium was replenished daily by adding aerated-water.

S. serrulatus (Koch, 1841) was collected from the lentic environs of Muriyad Wetland Kerala with the help of a tow net made of bolting silk of 70μm mesh size. The collection was taken to the laboratory in live condition and 20 healthy egg bearing females were sorted out under a stereo zoom microscope and were transferred into 5 liter glass aquarium, containing live *Chlorella* cells (2 x 10^6 cells ml^{-1}). A mild aeration was given to this stock culture and

Fig.1 Reproduction in Simocephalus serrulatus

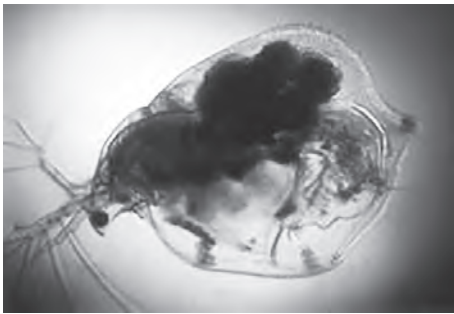

Fig.1a : Parthenogenetic female with eggs (1.726 mm)

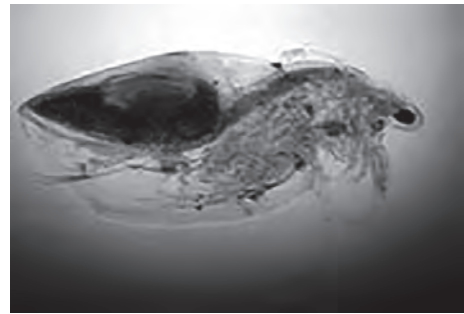

Fig.1b: Ephippial Female (1.454 mm)

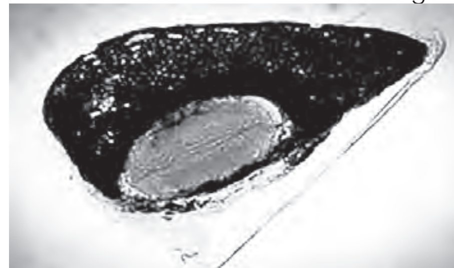

Fig.1c: Single ephippium with resting egg (0.982 mm)

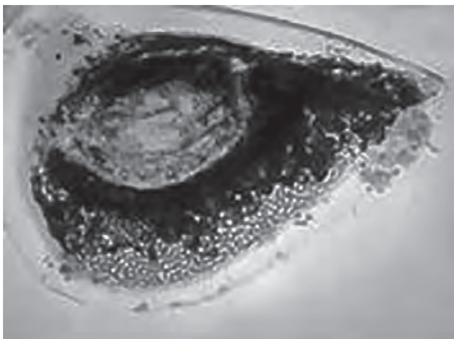

Fig.1d. Ephippium without resting egg (0.922 mm)

Fig.1e: Resting egg enlarged (0.392 mm)

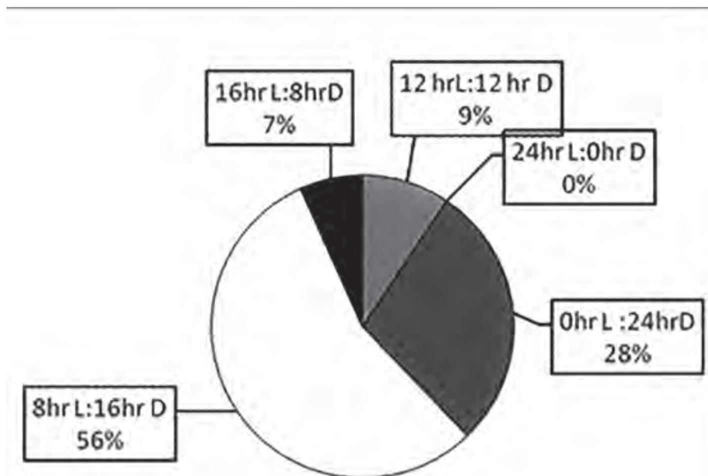

Fig.2. Pie diagram showing the Ephippial population in different photoperiods

was acclimatized for approximate 50 days before starting the experiments.

From this stock culture 3 replicates were prepared by putting 20 neonates each in separate glass jars of 5 liters capacity providing *Chlorella* density (2 x 10^6 cells ml^{-1}) and was exposed to 5 different photoperiod conditions (12 L: 12D; 24 L: 0D; 0L: 24D; 08 L: 16D; 16L: 8D) by keping under fluorescent light of 1000 lux for the specified light durations. The glass jars were maintained at temperature 26°C±2.0, pH 7.4±0.2 and Do_2 5.2±0.2. The non-ephippial forms (parthenogenetic) and ephippial females along with detached ephippia were collected from each tank, by taking a sample of 10ml using a wide mouthed glass pipette and counted it under a binocular stereozoom microscope Luxeo 4Z. The mean number of this population under each light exposure was expressed per liter.

After completion of experiment the contents in the aquarium was filtered using different sieves to isolate the ephippia containing the resting eggs to prepare an egg bank for future use. The sorted eggs were stored in dark containers inside the refrigerator.

Results and Discussion

The population developed during the laboratory culture comprised parthenogenetic females, males and ephippia bearing females. The ephippial females (Fig. 1b; mean size: 1.52 × 1.20 mm), smaller than parthenogenetic females (Fig. 1a; mean size: 1.720× 1.147 mm), and without the blunt posterior spine of carapace. The ephippial development was completed in duration of 65-70 hrs. The ephippial females were found to cast off their ephippia along with moult. The ephippium triangular (Fig. 1c; mean size: 0.973 × 0.648 mm), orange-yellow coloured, with honeycomb ornamentation, darkly pigmented with melanin, enclosing a single egg.

The newly released ephippia floated on the surface of the medium for some time and then sank to the bottom or get adhered to the side walls of the container. The egg distinctly orange coloured measured a mean size of 0.392 × 0.201 mm, and encased by an inner transparent and an outer thick leathery membrane (Fig.1e). Generally the development of egg and the ephippium most often took place simultaneously and the eggs were deposited into the ephippium. However, some of the ephippia were found to be produced without resting eggs within it (Fig.1d), this can be attributed to the absence of synchronization of egg release and ephippial development. In *Moina,* the eggs and ephippia develop simultaneously and the ephippial formation is not depended on the fertilization of the eggs[12].

The role of environmental factors in inducing ephippia formation has been studied by several workers. The ephippial production found to takes place when the food supply is low[8, 13, 14]. The crowding is found to influence the production of ephippial females in *C. cornuta* and *D. lumholtzi* [15, 16,17]. The present study is also in agreement with this observation (Table-1). The role of food scarcity and crowding as important factors regulationg ephippial production has earlier been indicated in *S. serrulatus* [18,19]. The respective percentage of ephippial production under different photoperiods is given in Fig. 2. The ephippial production was totally absent when exposed to 24 hour light. The percentage of ephippium production was lowerest under 16 hr. L (7%) and 12hrL (9%) respectively. The maximum ephippial production (56%) occurred at 16 hour darkness (Fig. 2). The decreased ephippial production under long periods of light exposure and increased production under prolonged darkness point out the role of this factor in influencing the formation of resting eggs in this species. The study

Table 1. The production of parthenogenetic females and ephippial females under different photoperiods

Photoperiod	12 hr. Light: 12 hr. Dark		24hr. Light: 0hr. Dark		0hr. Light : 24hr. Dark		8hr. Light : 16hr. Dark		16hr. Light: 8hr. Dark	
Mean Population per liter	Non-ephippial	Ephippial	Non-ephippial	Ephippial	Non-ephippial	Ephippial	Non-ephippial	Ephippial	Non-ephippial	Ephippial
10th day	116	0	0	0	112	0	146	0	248	0
20th day	140	0	140	0	210	0	646	184	1016	0
30th day	184	0	108	0	186	0	973	846	1086	0
40th day	1206	166	116	0	420	212	2108	2019	960	0
50th day	2628	1220	849	0	1680	1912	2884	3960	1420	840
60th day	610	180	1240	0	1420	2616	1672	2242	340	280
Total population	4884	1566	2453	0	4028	4740	8429	9251	5090	1120

indicate that along with other factors like crowding light exposure duration is also an important stimulus which influence the production of resting eggs in this species. Increased production of ephippia has been reported in a natural pond population of *S. vetulus* during winter[20]. This can be attributed to the influence of decreased photoperiod during winter months.

Conclusion

The results of the present study indicate that reduced light exposure duration is one of the environmental stimulus which control the ephippia formation and resting egg production in *S. serrulatus*. This will be helpful for the production of an egg bank of this species.

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Photo-decolorization potential of green synthesized titanium dioxide nanoparticles

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Abstract

Decolorization of textile dyes via photocatalysis is an area of growing interest these days due to the increasing demand to reduce pollution caused by them. Metal oxide nanoparticles are known to be potential photocatalysts, titanium dioxide nanoparticles (TiO_2-NPs) being one of them. Titanium dioxide (TiO_2) is a semiconductor material which possesses a special feature that it can use natural UV to appropriate separation between its valence and the conduction bands, which can be surpassed by the energy content by a solar photon. Thus, degradation of contaminants in wastewater using TiO_2 suspension is a very promising process. The present study is a significant report on the photocatalytic efficiency of green synthesized titanium dioxide nanoparticles (TiO_2-NPs) as an alternative for chemically synthesized photocatalysts for treatment of textile wastewater. Green synthesized titanium dioxide nanoparticles (TiO_2-NPs) showed potential photo-decolorization, with decrease in decolorization, with increasing dye concentration irrespective of the light source.

Keywords: TiO_2-NPs, titanium, nanoparticles, decolorization, sunlight, UV

Introduction

Nanoparticles have a broad range of physicochemical properties that make them particularly attractive as separation and reactive media for water purification[1]. Conventional techniques enable synthesis of large quantities of nanoparticles in less time but they use toxic chemicals as capping agents to maintain stability, which ultimately causes toxicity to environment. Thus, green nanotechnology is an eco-friendly and cost-effective alternative. Plant extract provide natural capping agents in the form of proteins. Photocatalytic nanomaterials allow ultraviolet light to destroy pesticides, industrial solvents and germs. NaP1 zeolites have been successively used to remove Cr (III), Ni (II), Zn (II), Cu (II) and Cd (II) from metal electroplating wastewater [2].

Compared to metal nanoparticles, metal oxide nanoparticles are popular for photocatalysts as shown by the various instances in literature, titanium dioxide being one of them. TiO2 doped with nitrogen ions or metal oxide serves as a potential photocatalyst under either visible or UV light[3]. The photocatalytic properties of titanium dioxide were discovered by Akira Fujishima in 1967 and published in 1972[4]. Solid TiO_2 absorbs light in the near UV (350 nm) causing an electron from the valence band to be excited across the band gap of +3.0 eV up to the conduction band containing free electrons[5].

The present study is novel in a couple of significant aspects. Firstly, there are not many reports on the photocatalytic efficiency of TiO_2-NPs synthesized via the green method. Besides there is not much literature available for TiO_2-NP-mediated photocatalysis of the chosen dye.

Materials and Methods

Plant collection and identification

The plants were collected from Pirayiri, Palakkad District, Kerala, and authenticated at Botanical Survey of India, Coimbatore, Tamil Nadu.

Synthesis of TiO_2-NPs

Aqueous leaf extract was prepared [6]. Titanium (IV) isopropoxide was purchased from Sigma, India. The precursor was prepared by mixing titanium (IV) isopropoxide with ethanol in the ratio 1: 10 by constant stirring for 1 h. The crude

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aqueous leaf extract and the precursor were taken in the ratio 1: 9 and subjected to stirring for 4 h at 50^0C. TiO_2-NPs were obtained by centrifugation at 10000 rpm for 15 mins, washed with ethanol and centrifuged again at 5000 rpm for 10 mins. The separated particles were dried and ground followed by calcination at 500^0C for 3 h [7, 8]. Elemental purity of the particles was confirmed using EDX (JEOL JEM 2100 high resolution transmission electron microscope).

Preparation of aqueous dye solution

The textile dye Blue RGB was purchased from local sources. Aqueous dye solutions of various concentrations (10, 20, 40, 60, 80 and 100 ppm) were prepared.

Analysis of photo-decolorization

Parameters were optimized for maximum decolorization-nanoparticle and nanocomposite dose, dye concentration, time and pH.

Effect of nanoparticle dose on dye concentration

Varying concentrations of TiO_2-NPs (2, 4, 6, 8, 10 and 12 mg) were added to the aqueous dye solutions (30 ml each) of various concentrations and subjected for activity under sunlight and UV irradiation with respective controls. Absorbance was noted for a period of 30 h at time intervals of 6 h and percentage of decolorization calculated. The dye concentration and, dose of nanoparticles, with maximum decolorization was selected for optimization of further parameters. Also, the time after which no significant change could be noted was used as the period for optimizing other parameters.

Effect of time on decolorization

The optimum dye concentration, and particle dose obtained in the previous experiments were used to observe the effect of time on decolorization, along with controls. They were subjected for decolorization under sunlight and UV irradiation for a period of 24 h and absorbance noted every 2 h for calculation of percentage of decolorization.

Effect of pH

Different pH solutions (3, 5, 6, 7, 9 and 11) of the optimum dye concentration were prepared and the optimum dose of particles was added. It was then subjected for decolorization under sunlight and UV irradiation. The absorbance was noted for 18 h at time intervals of 6 h. Percentage of decolorization was then calculated.

Results and Discussion

Plant collection and identification

The plants collected were identified as *Ocimum basilicum* L. var. *purpurascens* Benth belonging to the Lamiaceae family.

Synthesis of TiO_2-NPs

Characteristic creamish/pale white powder was obtained which showed resemblance to those obtained using *Nyctanthes arbor-tristis* L. leaf extract [7]. The formation of nanoparticles may have been facilitated by the presence of linalool [9, 10, 11]. Analysis via Energy Dispersive X-ray (EDX) spectrometers confirmed the presence of elemental titanium and oxygen signals of the TiO_2-NPs with their weight percentages being 49.70 and 50.30, respectively (Fig.1).

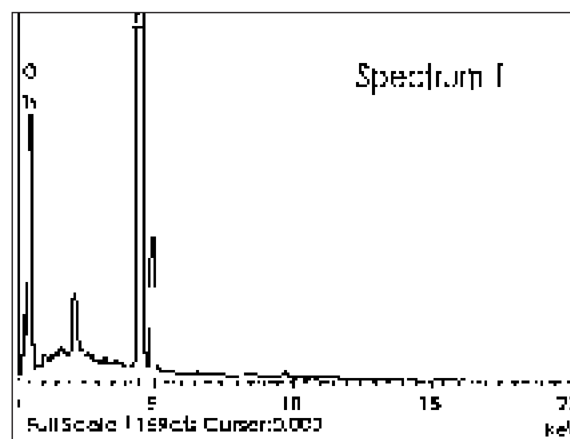

Fig.1. EDX spectrum of green synthesized TiO_2-NPs

Figures 2 and 3 showed that decolorization decreased with increasing dye concentration irrespective of the light source and 10 ppm was found to be the optimum concentration for maximum decolorization, with hardly any change after 24 h. Thus 24 h was selected as the running period for optimization of time.

Under sunlight, the percentage of decolorization was found to increase with increasing amounts of TiO_2-NPs up to 10 mg for the dye concentrations 10, 20, 40, 60, 80 and 100 ppm while 12 mg of TiO_2-NPs decreased decolorization. Under UV irradiation, the percentage of decolorization increased with

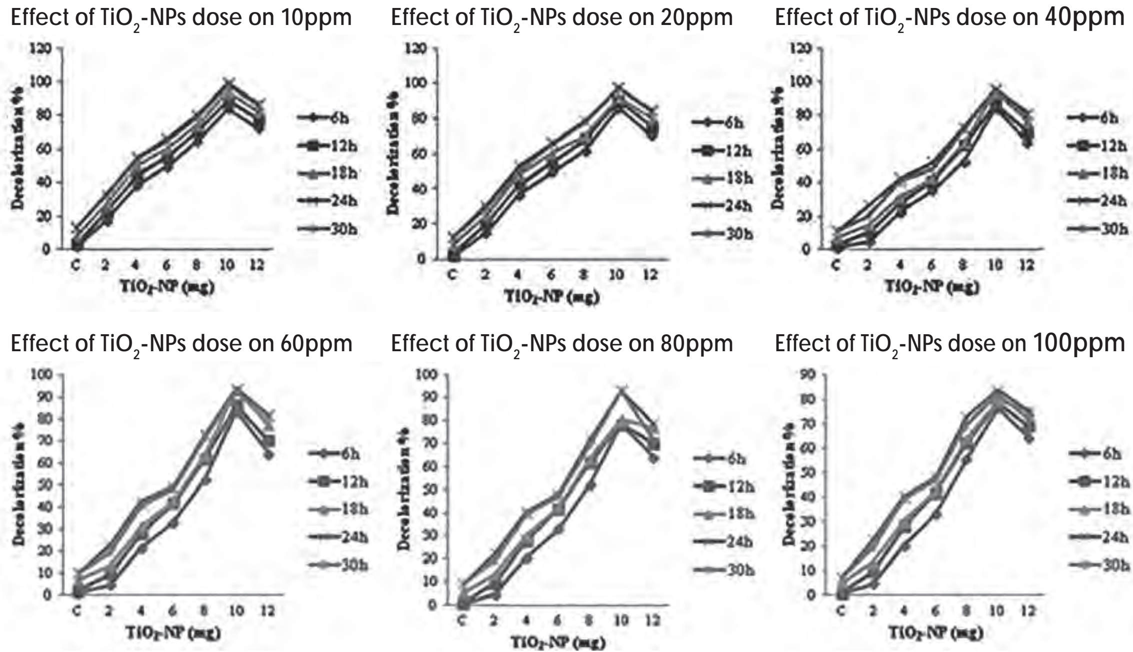

Fig.2. Effect of TiO_2-NPs dose on dye concentration on sunlight-mediated photocatalysis of the textile dye Blue RGB

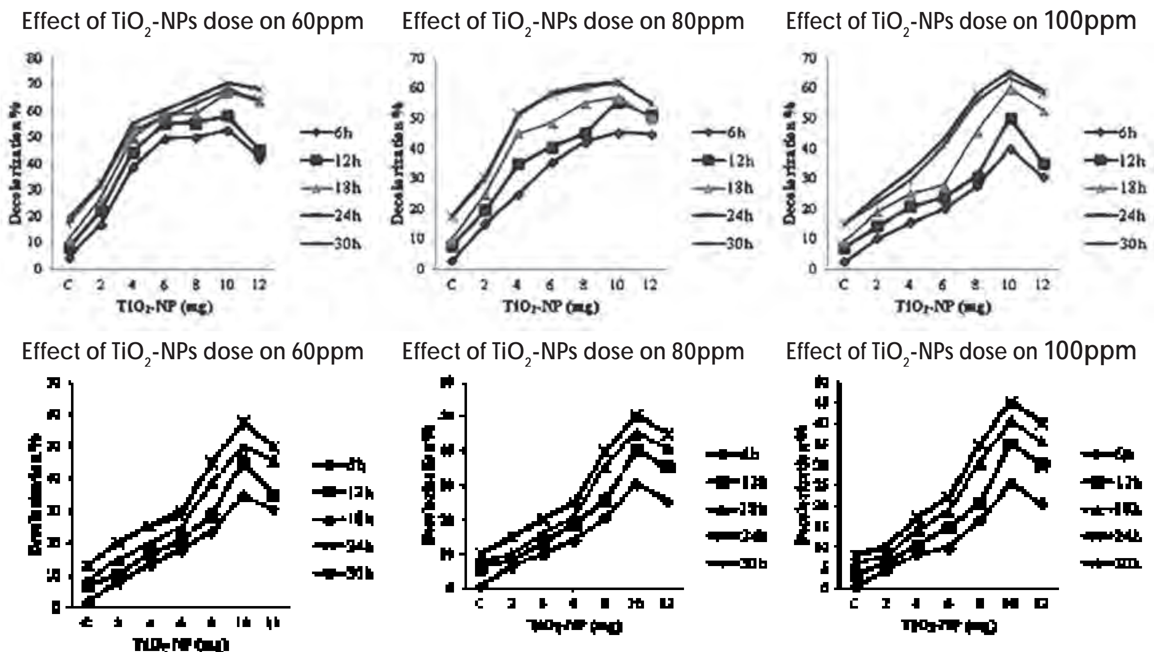

Fig.3. Effect of TiO_2-NPs dose on dye concentration on UV-mediated photocatalysis of the textile dye Blue RGB

increase in TiO_2-NPs for all concentrations with 10 mg being the optimum concentration. But the decolorization was better under sunlight. When effect of time was checked with 10 ppm aqueous dye solution and 10 mg TiO_2-NPs, it was observed that after 18th hour, there was hardly any change indicating that 18 h is the optimum time required for UV-mediated photocatalysis while for sunlight-mediated photocatalysis it was only 14 h (Fig.4). Figures 5 and 6 showed that under both sunlight and UV irradiation, the optimal pH is 6, which is the original pH of the dye. The observations for analysis of decolorization potential can be summarized as in Table 1.

Table1. Photo-decolorization of Blue RGB by green synthesized TiO_2-NPs

Light source	Sunlight	UV
Dye concentration	10 ppm	10 ppm
TiO_2-NP dose	10 mg	10 mg
Time	14 h	18 h
pH	6	6
Decolorization %	99.7	70.5

Anatase TiO_2-NPs are reportedly the best material with the highest photocatalytic detoxification and the ones with 5 nm size are best for the photocatalysis of dichloroacetic acid [12, 13]. The visible light absorbing chromophores of the dye absorb photons creating excited dye molecule. From the excited dye, the electron is injected into the conduction band of TiO_2, leaving cationic dye radical [14]. TiO_2 can efficiently photocatalyse Remazol Brilliant Blue R using artificial and sunlight radiation sources [15].

Conclusion

A common plant has been put to use for the synthesis of an efficient photocatalyst likeTiO_2-NPs bringing into light yet another use of the plant. Decolorization decreased with increasing dye concentration irrespective of the light source. While the amount of particles and dye concentration were

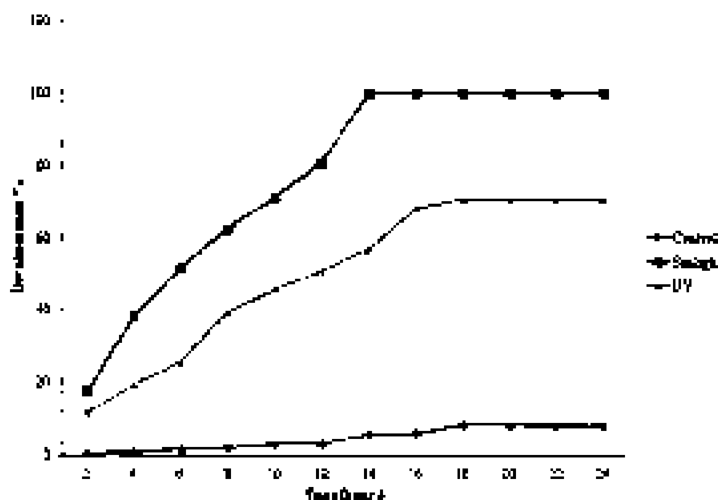

Fig. 4. Effect of time on decolorization of Blue RGB for TiO_2-NPs-mediated photocatalysis

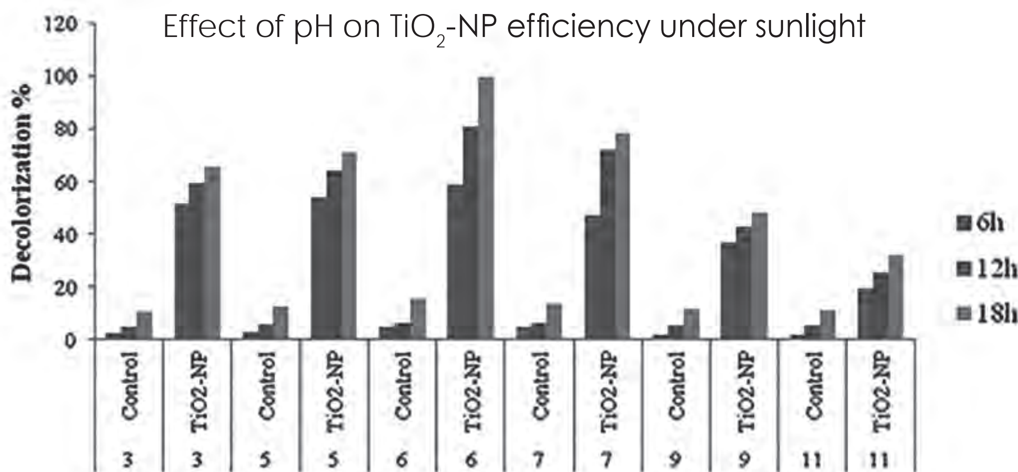

Fig. 5. Effect of pH on decolorization of Blue RGB for TiO_2-NPs + sunlight-mediated photocatalysis

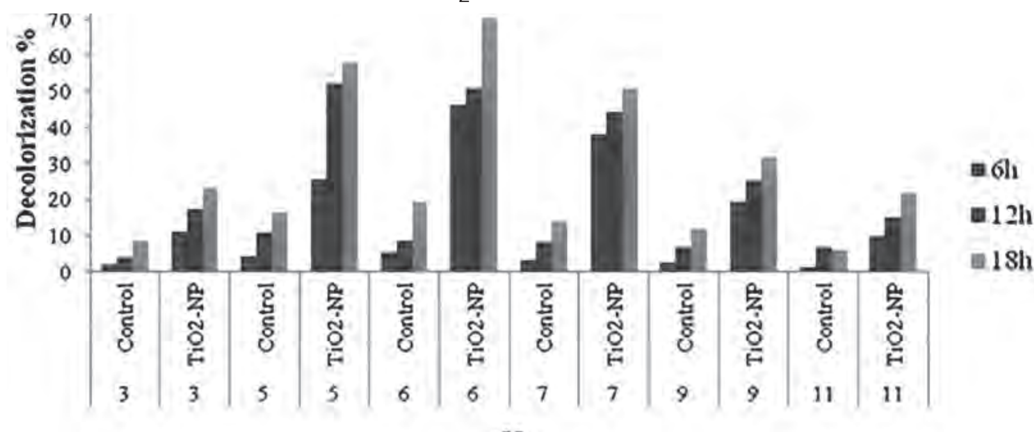

Fig.6. Effect of pH on decolorization of Blue RGB for TiO_2-NPs + UV-mediated photocatalysis

same, there was a vast difference in the percentage of decolorization and time depending on the light source. The study implies that cheap and easily available light source can be used to tackle waste treatment problems. Besides, commonly available plant sources can be used for the manufacture of photocatalysts.

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A preliminary study on Coleopteran diversity in the riparian habitat of Chaliyar River

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Abstract

The riparian habitat of Chaliyar River was extensively studied for the Coleopteran fauna as this order represents more species than any other order. Temporal diversity and abundance of three different sites were determined. The study period (October 2011 to July 2012) divided in to four different seasons' viz., the Post-monsoon, winter, summer and Monsoon. Sampling sites selected were upper, middle and lower region of Chaliyar River. The beetles were collected, preserved and identified in accordance with the standard scientific procedure. The collection methods include hand picking, sweeping and beating technique. A total of 7 families were recorded from the study area, they were Carabidae, Staphylinidae, Buprestidae, Coccinellidae, Tenebrionidae, Scarabaeidae and Chrysomelidae. Among the 7 families the genus level identification was done only for Coccinellidae, Tenebrionidae and Chrysomelidae. From these families 8 genus were identified, they are Epilachna (Coccinellidae), Gonocephalum (Tenebrionidae), Aulacophora, Altica, Aspidomorpha, Chiridopsis, Dicladispa and Cryptocephalus (Chrysomelidae). Two specimens from Chrysomelidae family were identified up to species level, ***Chiridopsis bipunctata*** and ***Dicladispa armigera***. Out of the 7 families, Chrysomelidae was the dominant one comprising 6 genus and 2 species. The most abundant family was Chrysomelidae and least abundant were Staphylinidae, Buprestidae and Scarabaeidae. The families which represents all seasons were Carabidae, Coccinellidae, Tenebrionidae and Chrysomelidae. The high abundance of beetles belongs to Chrysomelidae and Coccinellidae at the middle Chaliyar is due to the presence of agricultural practice. A wide variety of herbs, shrubs and trees cause high abundance of leaf beeltes and ladybird beetles.

Key words: Chaliyar, Riparian habitat, Coleoptera, Temporal diversity, Temporal abundance

Introduction

Coleoptera is an order of insects commonly called *beetle*. It contains more species than any other order, constituting almost 25% of all known life-forms and about 40% of all described insect species, about 400,000 species[1], and new species are discovered frequently[2] . The largest taxonomic family, the Curculionidae, also belongs to this order. Coleoptera classified into four suborders (*Adephaga, Archostemata, Myxophaga,* and *Polyphaga).* The adaptive radiation of angiosperms helped drive the diversification of beetles[3,4,] as four of the six mega diverse families of beetles are primarily angiosperm-feeders: *Curculionidae, Chrysomelidae, Scarabaeidae,* and *Cerambycidae.* Coleoptera are found in nearly all natural habitats, including freshwater and marine habitats[5], everywhere there is vegetative foliage, from trees and their bark to flowers, leaves, and underground near roots—even inside plants in galls, in every plant tissue, including dead or decaying ones.

Diversity and abundance of the coleopteran fauna from organic and conventional management systems in southern England, revealed that in total, 27 749 individuals from 140 coleopteran species were identified and recorded during the 8-week study period[6]. Carabid beetles formed the dominant group of epigeal Coleoptera in pitfall trap catches. Cloud forest dung beetles (Coleoptera: *Scarabaeinae*) in the Western Ghats, a global biodiversity hotspot in South Western India; and the time comprehensive data on the community structure, species compositional endemism of dung beetle assemblage in a tropical montane cloud forest (TMCF) from South Asia were indicated the importance of Coleopterans at Western Ghat[7]. Among the four oriental genera of the tribe Helluonini, *Omphra* Dejean (Coleoptera: *Carabidae*), is unique for its endemism to the Indian subcontinent and aptery. The present study was undertaken to determine the seasonal diversity and the abundance of Coleopterans at the riparian habitat of Chaliyar River.

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Materials and Methods

Chaliyar is one of the west flowing rivers originating from the Western Ghats and experiences three different seasons, the north east monsoon from September to December, dry season from January to May and the south west monsoon during June to August. Altitudinal gradient ranges from 11M to 2066 M above mean sea level. Chaliyar, one of the major rivers of Kerala, originates from the Ilambalari (Elembalai) Hills in Gudalur of Nilgiris District (Tamil Nadu). Chaliyar River is a combination of Korapuzha, Kallai, and Chaliyar. This area gets an average annual rain fall of 3800mm. The river basin covers a wide variety of habitats ranging from the Montane Grasslands, Shola Forests, Evergreen Forests, Semi-evergreen Forests, Moist Deciduous Forests, Wetlands and human habitations. The study area includes Upper region (Chaliyarmukku), Middle region (Vettupara) and Lower region (Feroke) of Chaliyar River.

Upper region: Chaliyarmukku is situated in the upper stream of Chaliyar River comprises dense forests with splendid variety of flora and fauna, located 18 km away from Nilambur. This forest ecosystem provides a humus rich environment that supports a preferable habitat for diverse group of organisms. The area is an integral part of Nilgiri Biosphere reserve.

Middle region: Vettupara is in the middle streams, which are near to human inhabitance. People use this tributary of Chaliyar for various domestic purposes. There occur various agricultural practices and the land used for grazing by domestic animals.

Fig. 1 Habitat photo of Upper region

Fig.2 Habitat photo of middle region

Fig. 3 Habitat photo of Lower region

Lower region: Feroke is located in the downstream of Chaliyar. This area is also situated near to human settlements and people use the water and land for various purposes. It is an area which spreads over about 24 hectares of mudflats. This mud is formed by the deposition of silt by the Chaliyar River.

For the study the beetles were collected, preserved and identified. The collection methods include hand picking, sweeping (50-60 cm long strong handle with 50 cm depth bag is used) and beating technique. The preservation method includes pinning and preservation in 70% ethyl alcohol. The identification methods were by using analytical keys [8,9,10,11,] and by experts opinion of Dr. Raj Mohana from ZSI, Dr. Nasar from Calicut University and Mr. Mohammed Shameem from College of Agriculture, Trivandrum and by comparing with pictures and descriptions.

Results and Discussion

A total of 7 families were recorded from the study area, they are Carabidae, Staphylinidae, Buprestidae, Coccinellidae, Tenebrionidae, Scarabaeidae and Chrysomelidae (Table.1). Among the 7 families the genus level identification was done only

for Coccinellidae, Tenebrionidae and Chrysomelidae. From these families 8 genus were identified, they are Epilachna (Coccinellidae), Gonocephalum (Tenebrionidae), Aulacophora, Altica, Aspidomorpha, Chiridopsis, Dicladispa and Cryptocephalus (Chrysomelidae). From the family Chrysomelidae two specimens were identified up to species level, *Chiridopsis bipunctata* and *Dicladispa armigera.* By the analysis of different site records the data reveals that in site-1 (Table.3) five families, in site-2 (Table.4) only three families and in site-3 (Table.5) six families were recorded. Chrysomelidae is the dominant family comprising 6 genus and 2 species were identified and constituting 80% of total beetle population. Among beetles some are pests (*Dicladispa armigera*) and some are beneficial (Lady bird beetles and Dung beetle). So they have a remarkable role in agriculture.

The families recorded in all seasons are Carabidae, Coccinellidae, Tenebrionidae and Chrysomelidae (Table.2). The families recorded only in monsoon are Buprestidae and Scarabaeidae. The family which is not recorded only in post-monsoon is Staphylinidae. The most abundant family was Chrysomelidae (80%) and least abundant are Staphylinidae, Buprestidae and Scarabaeidae (1% each) (Fig.1). The family diversity is more in site-3(6 families) and least in site-2 (3 families) but the abundance is more in site-2. Only Chrysomelidae family recorded in three sites. Images of identified beetles were presented in Fig.2.

The high abundance of beetles (Chrysomelidae and Coccinellidae) at the middle Chaliyar is due to the presence of high agricultural practice. A wide variety of herbs, shrubs and trees cause high abundance of leaf beeltes and ladybird beetles there. Because some are pests (Dicladispa armigera) and some are beneficial (Lady bird beetles, Dung beetle). As predators of invertebrates, including many pests, most ground beetles are considered beneficial organisms. The relative abundance of various families recorded during the study can be represented as Chrysomelidae> Coccinellidae> Tenebrionidae> Carabidae> Staphylinidae= Buprestidae= Scarabaeidae.

Studies of Coleopteran fauna of Kerala are limited. The present study revealed the Coleopteran diversity and abundance in three study areas of Chaliyar and compared the diversity according to seasonal variation. The information on the Coleopteran fauna of these areas is nil. Hence a serious attempt was made to study these aspects. A study was conducted by Sabu *et al.*[7], on Cloud forest dung beetles (Coleoptera: Scarabaeinae) in the Western Ghats. Among the four oriental genera of the tribe Helluonini, *Omphra* Dejean (Coleoptera: Carabidae), is unique for its endemism to the Indian subcontinent.

Table. 1 List of Coleopterans recorded from the study area.

Order	Sub-order	Family	Common Name	Genus	Species
Coleoptera	Adephaga	Carabidae	Ground Beetle		
	Polyphaga	Staphylinidae	Rove Beetle		
	Polyphaga	Buprestidae	The flat headed Borers		
	Polyphaga	Coccinellidae	Ladybird Beetle or Lady Beetle	Epilachna	
	Polyphaga	Tenebrionidae	Darkling Beetle	Gonocephalum	
	Polyphaga	Scarabaeidae	Scarabs or Lamellicorn Beetle		
	Polyphaga	Chrysomelidae	Leaf Beetle	Aulacophora Altica Aspidomorpha Chiridopsis Dicladispa Cryptocephalus	bipunctata armigera

Table. 2 Checklist of the Families recorded in different seasons.

	Season	Post-monsoon	Winter	Summer	Monsoon
	Month	October to December	January to February	March to May	June to July
Families	Carabidae	+	+	+	+
	Staphylinidae	–	+	+	+
	Buprestidae	–	–	–	+
	Coccinellidae	+	+	+	+
	Tenebrionidae	+	+	+	+
	Scarabaeidae	–	–	–	+
	Chrysomelidae	+	+	+	+

Most adult and larval beetles are herbivores or predators of other insects. Some beetles are scavengers on decomposing organic matter. So contribute to the recycling of nutrients essential for future production. Usually adults and larvae of the same species have the same feeding habits, eg. both are phytophagous. A wide range of beetles is of economic importance since they interfere with agricultural and forestry crops, timber products, stored products, etc. However, beetles do not transmit any diseases of man or livestock. Due to the fact that there are many predators, herbivores and scavengers amongst the beetles, they play an important role in maintaining the ecological balance in natural systems. Furthermore, many host-specific species are in use as biocontrol agents of insect pests

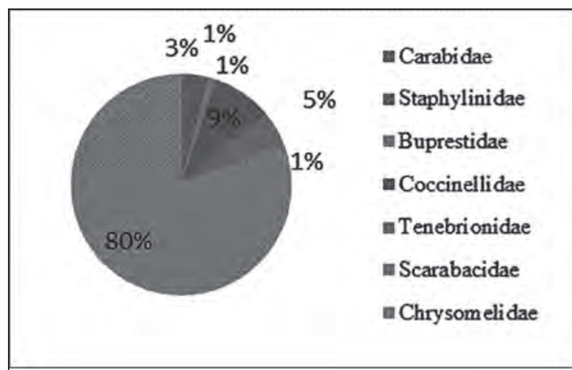


Fig.1 Diversity of families in the study area.

Table. 3 List of coleopterans in site-1

Sl. No.	Family	Genus
1	Carabidae	
2	Staphylinidae	
3	Tenebrionidae	Gonocephalum
4	Scarabaeidae	
5	Chrysomelidae	Dicladispa

Table. 4 List of Coleopterans in site-2

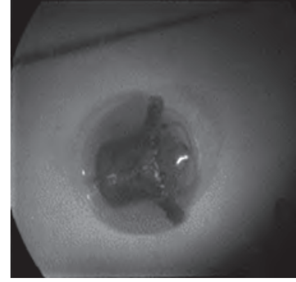
Sl. No	Family	Genus
1	Buprestidae	
2	Coccinellidae	Epilachna
3	Chrysomelidae	Aulacophora Altica Aspidomorpha Chiridopsis Cryptocephalus

Table. 5 List of Coleopterans in site-3

Sl. No	Family	Genus
1	Staphylinidae	
2	Buprestidae	
3	Coccinellidae	Epilachna
4	Tenebrionidae	Gonocephalum
5	Scarabaeidae	
6	Chrysomelidae	Aulacophora Altica

Epilachna species

Gonocephalum species


Aspidomorpha species

Cryptocephalus species.

Chiridopsis bipunctata

Dicladispa armigera.

Carabidae

Staphylinidae

Chrysomelidae

Fig.2 Images of Beetles identified

and noxious weeds. Apart from this, many beetles are important for the pollination of plants, mainly by means of walking on smaller, inconspicuous flowers. Some have a negative impact by competing for food resources or damaging products.

The family diversity is more in Feroke but the abundance is more in Vettupara. Only Chrysomelidae family recorded in three sites. Leaf beetles are more in the Vettupara region because it is a public place and agricultural practices are done by local people, it may be the reason for their high abundance and diversity. Beetles play a remarkable role in agriculture, because some are pests and some are beneficial to human by controlling the pests and by other ways. Although they are widely distributed, they are affected human actions and habitat stability. Their population is affected by deforestation, urbanization, etc. Monsoon is the season which attracted all the families of Coleoptera. Out of the seven families studied, four were present throughput the season. But Scarabeidae and Buprestidae were appeared only during the monsoon and Staphylinidae were absent only during post monsoon period. Chaliyar is blessed with rich biodiversity of Coloeptera of which only a small portion is able to collect and identified at species level. This is a first attempt of that kind and need further extensive studies in this field.

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A checklist to the vascular flora of Anamudi Shola National Park, Munnar, Idukki district, Kerala

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Abstract

A detailed research expedition was conducted in *Anamudi Shola National Park* (ASNP), situated in the Munnar Forest Division of Idukki District, Kerala State, for about one and a half decades, on the floristics, ethnobotanical and ecological aspects. About 674 taxa of vascular plants, which include 598 taxa of flowering plants and 76 pteridophytes, could be collected from the park. Flowering plants belonged to 356 genera under 111 families, out of which, Dicotyledons were represented by 484 taxa and Monocotyledons by 114 taxa. Pteridophytes belonged to 46 genera under 24 families. Among the 484 dicot taxa, 191 (39.5%) belonged to herbs, 163 (33.7%) were shrubs 130 (26.9%) were trees. Among the 76 pteridophytes, terrestrial taxa constituted 55%. Distribution of more than 30% of the plants is found to be restricted to Western Ghats. About 16% of plants were common to South India and Srilanka. Indo-Malesian species constituted about 8%. About 3% of plants were of Southeast Asian distribution and nearly 5% were exotics. Among angiosperms, 222 (37%) were endemics. Among the pteridophytes, out of the 76 taxa, only 4 taxa (5.2%) were found to be endemic. About 128 species (25%) classified as '*rare*' and another 26 species which were considered to be in a '*threatened state*' were reported from the park. Four species of angiosperms, earlier documented as '*Possibly extinct*' could also be rediscovered. This include *Arisaema attenuatum* Barnes & C.E.C. Fisch., *Arisaema psittacus* Barnes, *Pimpinel la pulneyensis* Gamble, *Symplocos monantha* Wight. About 66 plants were reported for the first time from Kerala as a result of this study. This include 44 angiosperms and 12 pteridophytes. The 76 pteridophytes collected from the park forms 23% of the total pteridophyte flora of the State (331 species). The significance of this figure becomes clear when we compare the small area of park (33.45 km^2) to the total area of the State of Kerala as a whole (38,863 km^2). From the study it becomes clear that Anamudi Shola National Park is one of the most diverse ecosystems in the Western Ghats, which is considered as one of the 'hottest' of the hotspots of biodiversity. The study also points out the need for conserving other Shola forests regions which are floristically and ecologically unique and highly important in all respects, before they get destroyed due to degradation, depletion or conversion of natural vegetation.

Keywords: Vascular flora, Anamudi Shola National Park, Munnar, Idukki, Kerala

Introduction

Anamudi Shola National Park (ASNP) which was declared as a National Park in December 2003 is located on the north-eastern part of the High Ranges of southern Western Ghats. It's geographical boundaries falls in the Marayur Forest Range of Munnar Forest Division, Idukki District, Kerala. The park consists of three Reserve Forests namely *Mannavan Shola* Reserve No. 58, *Pul laradi Shola* Reserve No. 57 and *Idivara Shola* Reserve No. 56. Together they occupy an area of 33.45 km^2 (as per SOI topo sheets) where as the notified extent is only 7.50 km^2 (Management plan, 2009-2018).

The ASNP represents a large number of plants and animals unique to the high altitude shola grassland vegetation. The giant fern, *Cyathia crinita* reaching upto 6 M height is a unique feature of Mannavan Shola. The Park provides the only source of water not only for the tribal hamlets within, but also for the inhabitants of *Kanthal lur, Puthur, Perumala, Pazhathottam* and *Silandiyar*. The Park also has unique features and provides ample academic opportunities for studying the biodiversity of montane vegetation and the ecological roles associated with the ecosystem. It can also serve as a field laboratory for activities like conservation education, research and monitoring and participatory management.

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Very limited information on the floristic elements of the shola forests were found in the *Working Plans* and *Management Plans*, the working documents for Forest Divisions. Much floristic information on the vegetation type is found in a fragmented state in earlier floristic works like Wight's[1] (1840-1853) *Icones Plantarum Indiae Orientalis*, Hooker's[2] (1872-1897) *Flora of British India*, Gamble and Fischer's[3] (1915-1936) *Flora of the Presidency of Madras*, and other regional and local floras. However, a flora particularly devoted to any of the shola forests is lacking.

Perhaps the first monographic floristic account on the shola forests was that of Fyson's[4] (1915-1921) *Flora of the Nilgiri and Pulney Hil l-tops,* while floristic particulars of other kinds of vegetations also occupy much coverage here. He also published another monumental work *The Flora of South Indian Hil l Stations*[5] in 1932, which covered a still broader area. The flora and vegetation of the shola forests of *the Nilgiris, Pulneys* and *Kodaikanal* in Tamil Nadu were relatively well studied[6-12] in comparison with that of Kerala.

Very few studies have been conducted in the shola forests of Kerala, parhaps due to their remote occurrance and unfavourable climates. A comparable floristic account of the shola forests of Kerala is due to the pioneering studies of Sebastine and Vivekananthan[13], Shetty and Vivekananthan[14-18,19,20-22] and others. Shetty and Vivekananthan could record a total of about 400 species from the *Anamudi* and surrounding regions, of which 143 are from grasslands alone. In addition to this, they described eight new taxa and about 28 rare and endangered species from the area. Rice[23], Jose *et al.*[24], Karunakaran [25] and others have added further documentation on the plant wealth of the high altitude shola-grassland regions. But most of these studies were concentrated mainly on the ecological aspects. In spite of these studies, botany of the region remained scanty. Later, Swarupanandan *et al.*[26] conducted some studies on the Floristic and Ecological aspects of the shola forests of the High Ranges of Idukki district, and the author who was the research scholar in this research project later took up this work for his doctoral dissertation works[27].

It is to be noted that majority of the shola forest regions in the state of Kerala is concentrated in the Idukki district, the largest and the most forested district of the state. But unlike other districts of Kerala, the flora of Idukki has not yet been completely exploredbecause of the peculiar nature of the terrain of this area which occupy both the low lands of the Deccan plateau at one end and the highest peak south of Himalayas (Anamudi) on the other. Thus, the available scattered and fragmentary floristic studies of the district were not enough to elucidate the actual picture of the floristic structure of this highly undulating area. It was Beddome (1882), who made the first botanical collection from the district, from Peermade area. Barnes, Beddome, Bourdillon, Meebold and Venkoba Rao made collections in the late 19th and early 20th century and their collections were cited by Hooker[2] and Gamble[3].

Mannavan Shola, which forms the major part of ANSP is the type locality of several species including *Arisaema psittacus* which was rediscovered by Nair *et al.*[28]. Barnes[29] studied the *Geraniaceae* of the High Ranges. Rama Rao[30] in his *Flowering Plants of Travancore* cited several places of this area. Iyppu[31] and Chandrasekharan[32] studied the forest types of this district. Sebastine and Vivekananthan[33], and Shetty and Vivekananthan[34,35] published brief accounts on the flora of Anamudi and Devikulam. Vivekananthan[36] studied the vegetation of Periyar Tiger Reserve and also enumerated 12 rare and threatened plants of the area. Studies by Shetty and Vivekananthan[37-43], Sharma *et al.*[44], *Nayar*[45], Sreekumar *et al.*[46,47], Nair and Sreekumar[48], and Pandurangan and Nair[49] resulted in the discovery of several new taxa from the district. Nagendran *et al.* [50] studied the *Podostemaceae*, Bhaskar[51] and Bhaskar and Razi[52,53] studied the *Balsaminaceae*. Mohanan *et al.*[54] reported some rare and interesting plants from the Idukki Hydro-electric Project Area. Balasubramanyam *et al.*[55] listed out some rare and interesting plants from the Pooyamkutty Hydro-electric Project Area. Jomy Augustine[56] conducted study on the angiosperm flora and the ethnobotany of Periyar Tiger Reserve and Rajesh[57] on the pteridophyte flora.

Detailed studies on the shola forests gained momentum during the last decade with the funding of several projects sanctioned to *Kerala Forest Research Institute*, Peechi by the Kerala Forest Department. First among them was on the floristic and ecological aspects done by Swarupanandan *et al.*[58] on the sholas of Idukki district. Kishore kumar[27] continued these studies and conducted detailed floristic explorations in Anamudi Shola, Pambadam Shola and Eravikulam National Parks and also

extended his studies to other regions of Idukki district, and to some sholas of Wayanad regions as well. This resulted in more than a dozen of publications on the floristic, ethnobotanical and ecological aspects of the shola forests of the state[58-60,27,61-76].

The First Management Plan of the ANSP National Park which was published in 2009, enlists some of the species reported from this park as Annexures, though these lists are scanty (62 trees, herbs and shrubs 174, climbers 39). It is in this context, this detailed work gains its importance in the planning of future management of the park.

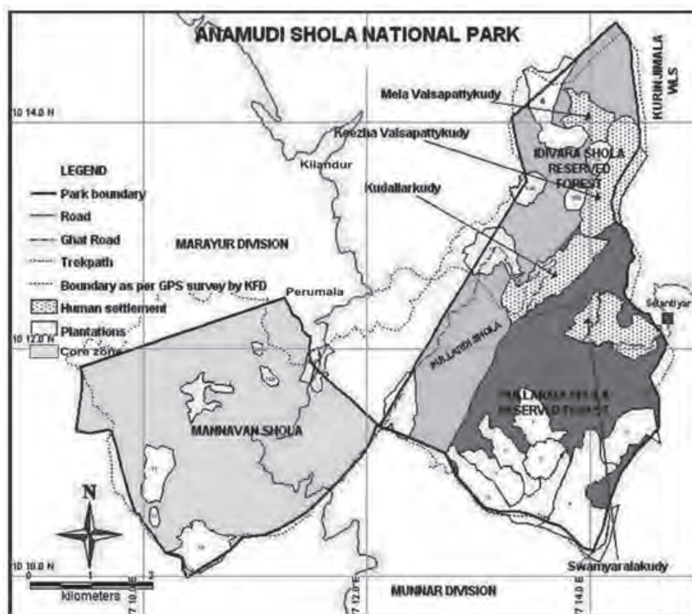

Fig.1 Map of ASNP showing *Mannavan* Shola, *Pullaradi* Shola and *Idivara* Shola, plantations and human settlements

This study consolidated the results of the detailed research expeditions conducted in this area for about one and a half decades on the floristics, ethnobotanical and ecological aspects.The work was started as a research project funded to *Kerala Forest Research Institute*, Peechi (KFRI/220/94)[68] by the *Kerala Forest Department* during 1994 and later continued as a part of the doctoral dissertation studies of the author.[27]

Materials and Methods

Study Area

The ASNP park falls in the *Kanthallur* and *Vattavada* Panchayats of *Devikulam* Taluk of Idukki District and includes three distinct but typical sholas, viz. *Mannavan Shola*, *Pullaradi Shola* and *Idivara Shola*. of which Mannavan shola is the largest in South India. On the northern side, the Park is bordered by the forests of *Marayoor Sandal Division* and a part of *Chinnar Wildlife Sanctuary*. The eastern side is bordered partly by the *Kurinjimala Sanctuary*. On the southern and western side are the forests *of Kannan Devan Hills* and the tea estates (Fig. 1). The Park provides ecological connectivity between the *Anamalai Tiger Reserve*, proposed *Palni Hills National Park* and the forests of *Kannan Devan Hills*.

The terrain is undulating with hillocks of varying heights. The altitude ranges between 1600-2400 M. The forest is seen as a continuous patch from 1600 to 2000 M, above which are small shola patches dispersed among the grasslands that are planted up with wattle. The area is traversed by small streams which drain into the Pambar River and Thalanchiyar to reach Amaravathi Reservoir in Tamil Nadu.

From 1994 onwards, regular field trips were organized to *Mannavan Shola, Idlimottai Sholas* and *Pullaradi Shola* regions for collecting plant specimens. Repeated collections were made for certain tree specimens to get both the flowering and fruiting specimens or the male and female flowers of the plant. Usually 4 specimens of each species were collected from all available habitats to study the range of variation. Collections were brought to the field stations and herbaria prepared as per standard procedure (Fosberg and Sachet[69]; Bridson and Forman[70]). Flowers and fruits of almost all specimens or even the whole plants (if small) were preserved either in *Formalin Aceto Alcohol* (FAA) or *Kew mixture* (9 parts of 70 % Ethyl alcohol, ½ part of 40% Formaldehyde and ½ part of conc. Acetic acid) for further microscopical studies. Large fruits were dried and poisoned separately to keep the shape unchanged. All the herbarium specimens were deposited in *Kerala Forest Research Institute Herbarium* (KFRI), Peechi.

The specimens were critically studied and identifications were made initially with the help of the *Flora of the Presidency of Madras*[3], *Flora of British India*[2] Fyson's *Flora of the Nilgiri and Pulney Hilltops*[4], *Flora of South Indian Hill Stations*[5], *Flora of Palni Hills*[11]. Illustrations of the species available

in Wight's *Icones Plantarum Indiae Orientalis*[1], both the works of Fyson[4,5] and those in *Illustrations of Palni Hills*[71] and the *Supplementary Illustrations of Palni Hills*[72] were referred for identification. Available monographs and revisions were also consulted. Several literature on phytogeographical studies and the internet cites of IPNI (*www.ipni.org*) were also referred to get the world distribution of each species. Specimens were also taken to the *Madras Herbarium* (MH) at Coimbatore and the *Calicut University Herbarium* (CALI) for verification and confirmation of identity. Those which needed further confirmation, were referred to experts in the concerned groups in India and abroad.

Since pteridophytes form a major part of the flora of shola forests, systematic collection of the pteridophytes was also done. Most of the plants were collected in full including the rhizomes by uprooting them. For larger plants and tree ferns, only the parts necessary for identification such as fronds including stipes, sporophylls with sporangia, rhizome scales, etc. were collected. Data on the habit, habitat and other ecological details were noted down to facilitate identification. Identification of the specimens was done mainly using pertinent literature such as Beddome[73-77], Manickam[78-80], Manickam and Ninan[81], Manickam and Irudayaraj[82], Nayar and Geevarghese[83], various monographs, M. Phil. and Ph. D. dissertations and hundreds of other publications on pteridophytes. Doubtful specimens were sent to experts and their identity got confirmed.

Results and Discussion

The plants collected are enumerated here according to the classification of *Bentham and Hooker*[84] and in accordance with the ICBN[85]. The genera and species under each family are arranged in alphabetical sequence. The correct name of the species is given in italics followed by the author citation. Local names and ethnobotanical importance (EBIS), if any are given in brackets. Details on ethnobotanical aspects are available in Kishore kumar *et al* [57, 58] and Kishore kumar and Sasidharan[61]. For Pteridophytes, family concept and classification of Pichi-Sermolli[86] was adopted and the species were enumerated as mentioned above.

I. ANGIOSPERMS: DICOTYLEDONS

1. *Ranunculaceae*

   Anemone rivularis Buch.- Ham. *ex* DC.

   Clematis munroiana Wight.

   Clematis smilacifolia Wall.

   Clematis wightiana Wall. *ex* Wight & Arn.

   Ranunculus reniformis Wall. *ex* Wight & Arn.

   Ranunculus wallichianus Wight & Arn.

   Thalictrum javanicum Blume.

2. *Magnoliaceae*

   Michelia nilagirica Zenk. - (Chembakam)

3. *Menispermaceae*

   Cocculus laurifolius DC. - (Marpingi, Aadukolli - EBIS)

   Stephania japonica (Thunb.) Miers - (Pasi chedi)

4. *Berberidaceae*

   Berberis tinctoria Lesch. - (Kozhikkal Mullu)

   Mahonia leschenaultii (Wall. *ex* Wight & Arn.) Takeda *ex* Gamble - (Manjanathi – EBIS)

5. *Brassicaceae*

   Cardamine africana L.

   Cardamine hirsuta L.

   Coronopus didymus (L.) Smith

6. *Violaceae*

   Viola betonicifolia Smith, subsp. *nepalensis* (Ging.) Becker

   Viola pilosa Blume

7. *Flacourtiaceae*

   Casearia thwaitesii Briq.

   Casearia zeylanica (Gaertn.) Thw.

   Hydnocarpus alpina Wight - (Vila Maram, Marotti, Vilan - EBIS)

   Scolopia crenata (Wight) Clos. - (Mullu Kara, Karim Kara - EBIS)

8. *Pittosporaceae*

   Pittosporum neilgherrense Wight & Arn.

   Pittosporum tetraspermum Wight & Arn.

9. *Polygalaceae*

   Polygala arillata Buch.-Ham. *ex* D. Don, var. *chartacea* (Mukherjee) Giri

   Polygala persicariifolia DC.

   Polygala sibirica L.

10. Caryophyllaceae

Cerastium indicum Wight & Arn.

Drymaria cordata (L.) Willd. *ex* Roem. & Schult., subsp. *diandra* (Blume) Duke

Spergula arvensis L.

11. Portulacaceae

Portulaca oleracea L.

12. Hypericaceae

Hypericum japonicum Thumb. *ex* Murr.

Hypericum mysurense Heyne *ex* Wight & Arn. - (Avaram Kola - EBIS)

13. Clusiaceae (Guttiferae)

Garcinia gummi-gutta (L.) Robs., var. *papilla* (Wight) Singh - (Kodampuli - EBIS)

14. Theaceae (Ternstroemiaceae)

Eurya japonica Thunb. - (Koora Mar - EBIS)

Eurya nitida Korth. - (Koora Mar - EBIS)

Gordonia obtusa Wall. *ex* Wight & Arn. - (Chembarasan)

Ternstroemia gymnanthera (Wight & Arn.) Beddome

15. Malvaceae

Abelmoschus angulosus Wall. *ex* Wight & Arn. - (Kattu Sembarathi)

Sida rhombifolia L.

Urena lobata L.

16. Tiliaceae

Grewia umbellifera Bedd. - (Kokki Valli)

Triumfetta pilosa Roth - (Erumachappu)

17. Elaeocarpaceae

Elaeocarpus glandulosus Wall. *ex* Merr. - (Kotlam Pazham - EBIS)

Elaeocarpus munronii (Wight) Mast. - (Shiru Kodala)

Elaeocarpus recurvatus Corner - (Rudraksham - EBIS)

Elaeocarpus tuberculatus Roxb. - (Koduvasi Maram, Shembara Maram - EBIS)

18. Geraniaceae

Geranium nepalense Sweet

19. Oxalidaceae

Biophytum intermedium Wight

Biophytum sensitivum (L.) DC., var. *candolleanum* (Wight) Edgew. & Hook. f.

Oxalis corniculata L. - (Pulichan, Kottampulichan - EBIS)

Oxalis latifolia Kunth - (Pulichan)

20. Balsaminaceae

Impatiens balsamina L. - (Thottachinungi)

Impatiens campanulata Wight - (Thottachinungi)

Impatiens chinensis L.

Impatiens coelotropis Fischer

Impatiens cordata Wight - (Thottachinungi)

Impatiens cuspidata Wight & Arn.

Impatiens goughii Wight

Impatiens hensloviana Arn.

Impatiens herbicola Hook. f.

Impatiens latifolia L.

Impatiens munronii Wight

Impatiens parasitica Bedd.

Impatiens phoenicea Bedd.

Impatiens tangachee Bedd. - (Aattalanji)

Impatiens wightiana Bedd.

21. Rutaceae

Acronychia pedunculata (L.) Miq. - (Veruku Theeni, Kari Maram, Chakki Maram, Mavurinji - EBIS)

Atalantia wightii Tanaka - (Kattu Elumachan, Kara - EBIS)

Murraya paniculata (L.) Jack. 1820 - (Paanamar, Kattu Mulla, Palakanni, Vellakkal Thirukan - EBIS)

Toddalia asiatica (L.) Lam. - (Kantha Mullu, Kantham Kolunthu, Kurangu Mullu - EBIS)

Zanthoxylum tetraspermum Wight & Arn. - (Kokki Mullu)

22. Meliaceae

Aglaia apiocarpa (Thw.) Hiern

Chukrasia tabularis A. Juss. - (Chandana Vembu, Vella Nangu - EBIS)

Cipadessa baccifera (Roth) Miq. - (*Potti*).

Trichilia connaroides (Wight & Arn.) Bentv. - (Karimaram, Pili Maram, Kozhipenali - EBIS)

23. Icacinaceae

Apodytes dimidiata E. Meyer *ex* Arn.

Gomphandra coriacea Wight (Chotta, Shotta, Chakki Maram)

Nothapodytes nimmoniana (Grah.) Mabber. (Pulippacha – EBIS)

24. Aquifoliaceae

Ilex denticulata Wall. *ex* Wight - (Noola).

Ilex malabarica Bedd. - (Aattu Njaval).

Ilex walkeri Wight & Gard. *ex* Thw. - (Noola).

Ilex wightiana Wall. *ex* Wight, var. *peninsularis* Hook. f. - (Aattu Njaval, Parasal).

25. Celastraceae

Bhesa indica (Bedd.) Ding Hou, Blumea Suppl. 4: 152. 1958 - (Penali - EBIS)

Euonymus crenulatus Wall. *ex* Wight & Arn., Prodr. 161. 1834

Microtropis ramiflora Wight, Ic. t. 977. 1845

26. Rhamnaceae

Rhamnus wightii Wight & Arn. - (Kokku valli)

Sageretia hamosa (Wall.) Brongn. - (Kokki Mullu)

Zizyphus rugosa Lam. - (Kotta Mullu - EBIS)

27. Vitaceae

Cayratia pedata (Lam.) Juss. *ex* Gagnep.

Parthenocissus semicordata (Wall.) Planch.

Tetrastigma nilagiricum (Miq.) B. V. Shetty - (Pashala Kodi - EBIS)

28. Staphyleaceae

Turpinia cochinchinensis (Lour.) Merr. - (Kanali - EBIS)

29. Sapindaceae

Allophylus concanicus Radlk., var. *lanceolatus* Gamble

Dodonaea angustifolia L. f. - (Vrali - EBIS)

30. Sabiaceae

Meliosma pinnata (Roxb.) Maxim., subsp. *barbulata* (Cufod.) Beus. - (Thakiri - EBIS)

Meliosma simplicifolia (Roxb.) Walp., subsp. *pungens* (Wall. *ex* Wight & Arn.) Beus.

Meliosma simplicifolia (Roxb.) Walp., subsp. *simplicifolia,* Hook. f. - (Kolakkatta, Chengoini - EBIS)

31. Anacardiaceae

Nothopegia beddomei Gamble. - (Era - EBIS)

Nothopegia monadelpha (Roxb.) Forman. - (Era - EBIS)

32. Connaraceae

Connarus wightii Hook. f. - (Karinkodi - EBIS)

33. Fabaceae

1. *Subfamily: Faboideae*

Cajanus rugosus (Wight & Arn.) Measen

Canavalia cathartica Thouars

Crotalaria beddomeana Thoth. & Ansari

Crotalaria calycina Schrank

Crotalaria fysonii Dunn

Crotalaria ovalifolia Wall. *ex* Fyson

Crotalaria pallida Dryand.

Crotalaria scabrella Wight & Arn.

Crotalaria semperflorens Vent. - (Chunda - EBIS)

Crotalaria spectabilis Roth.

Crotalaria wightiana Grah. *ex* Wight & Arn.

Cytisus scoparius (L.) Link.

Derris brevipes (Benth.) Baker-(Karinkodi- EBIS)

Desmodium microphyllum (Thunb.) DC.

Desmodium repandum (Vahl) DC. - (Kattu Avam Poovu - EBIS)

Desmodium triflorum (L.) DC.

Desmodium uncinatum (Jacq.) DC. - (Moschio, Pottu Kola, Kolagu Kola - EBIS)

Dumasia villosa DC. – (Pullooran Charadu Kodi - EBIS)

Flemingia grahamiana Wight & Arn.

Flemingia strobilifera (L.) R. Br. *ex* Ait.

Indigofera cassioides Rottl. *ex*. DC. – (EBIS)

Indigofera spicata Forssk.

Lablab purpureus (L.) Sweet. - (Kattavara - EBIS)

Leptodesmia congesta (Wight) Benth. *ex* Baker

Neonotonia wightii (Wight & Arn.) Lackey

Parochetus communis Buch.-Ham. *ex* Don

Rhynchosia rothii Benth. *ex* Aitch.

Shuteria involucrata (Wall.) Wight & Arn.

Shuteria vestita Wight & Arn.

Smithia gracilis Benth.

Tephrosia tinctoria Pers.

Uraria rufescens (DC.) Schindl.

Vigna bourneae Gamble

Vigna grahamiana (Wight & Arn.) Verdc.

Vigna sublobata (Roxb.) Babu & Sharma

2. *Subfamily: Caesalpinioideae*

Cassia floribunda Cav. - (Kattu Konna - EBIS)

Cassia mimosoides L.

3. *Subfamily: Mimosoideae*

Acacia dealbata Link. - (Silver Wattle, Karuva - EBIS)

Acacia mearnsii de Wilde. - (Black Wattle, Karuva - EBIS)

Acacia melanoxylon R. Br. - (EBIS).

Archidendron clypearia (Jack) Nielsen - (Pulivaka - EBIS)

34. Rosaceae

Photinia integrifolia Lindl., var. *sublanceolata* Miq. - (Choluvan - EBIS)

Prunus ceylanica (Wight) Miq. - (Poochapiddukku, Vedipottan - EBIS)

Prunus persica (L.) Batsch. - (Pikkees - EBIS)

Rosa leschenaultiana Red. & Thory *ex* Wight & Arn. - (Kattu Roja - EBIS)

Rubus ellipticus Smith in Rees. - (Vella Cheechi - EBIS)

Rubus fairholmianus Gard. - (Eruma Cheechi, Vettila Mullu - EBIS)

Rubus niveus Thunb. - (Karim Cheechi - EBIS)

35. Saxifragaceae

Parnassia mysorensis Heyne *ex* Wight & Arn.

36. Crassulaceae

Kalanchoe grandiflora Wall. *ex* Wight & Arn.

37. Droseraceae

Drosera peltata Sm. in Willd. - (Koshukotti Pullu, Azhukanni - EBIS)

38. Haloragaceae

Laurembergia coccinea (Blume) Kan.

39. Myrtaceae

Eucalyptus globulus Labill. - (Eucali - EBIS)

Eucalyptus grandis Hill *ex* Maid. - (Grandis - EBIS)

Eugenia bracteata (Willd.) Roxb. *ex* DC.

Eugenia calcadensis Bedd.

Rhodomyrtus tomentosa (Ait.) Hassk. - (Kirattan - EBIS)

Syzygium caryophyllatum (L.) Alston - (Potti Njaval - EBIS)

Syzygium cumini (L.) Skeels - (Valiya Njaval - EBIS)

Syzygium densiflorum Wall. *ex* Wight & Arn. - (Pilla Njaval - EBIS)

Syzygium gardneri Thw. - (Venkal Njaval - EBIS)

40. Melastomataceae

Medinilla malabarica Bedd.

Memecylon flavescens Gamble

Osbeckia aspera (L.) Blume

Osbeckia aspera (L.) Blume, var. *wightiana* (Benth. *ex* Wight & Arn.) Trimen

Osbeckia leschenaultiana DC.

Osbeckia parvifolia Arn.

Osbeckia reticulata Bedd.

Sonerila grandiflora R. Br. *ex* Wight & Arn.

41. Lythraceae

Rotala indica (Willd.) Koehne

42. Onagraceae

Circaea alpinia L., subsp. *imaicola* (Asch. & Magn.) Kitam.

Oenothera laciniata Hill

43. Passifloraceae

Passiflora edulis Sims - (Thattu Pootan, Kodikkai, Poonakkai - EBIS)

Passiflora leschenaultii DC. - (Murumurukkan Kodi - EBIS)

Passiflora ligularis Juss. - (Sweet Granadilla - EBIS)

Passiflora mollissima (H. B. K.) L. H. Bailey - (EBIS)

44. Cucurbitaceae

Diplocyclos palmatus (L.) Jeffrey - (Pambu kodi)

Solena amplexicaulis (Lam.) Gandhi

Trichosanthes lobata Roxb. - (Kovan Kodi, Pepparal Kodi, Kattu Padavalam - EBIS)

Zehneria maysorensis (Wight & Arn.) Arn., var. *umbellata* (Chakravarthy) Kumari - (Musumusukkan Chedi – EBIS)

Zehneria scabra (L. f.) Sond.

45. Begoniaceae

Begonia malabarica Lam. - (Minukki - EBIS)

46. Apiaceae

Bupleurum distichophyllum Wight & Arn.

Bupleurum mucronatum Wight & Arn.

Bupleurum wightii Kozo-Polj.

Centella asiatica (L.) Urban - (Kuthirakkulambu Chedi, Vallara - EBIS)

Heracleum ceylanicum Gardn. *ex.* Clarke

Heracleum sprengelianum Wight & Arn.

Hydrocotyle javanica Thunb. - (Vaite Kala, Vella Vaite – EBIS)

Pimpinella candolleana Wight & Arn.

Pimpinella leschenaultii DC.

Pimpinella pulneyensis Gamble

Sanicula elata Buch.-Ham. *ex* D. Don

Vanasushava pedata (Wight) Mukh. & Const.

47. Araliaceae

Pentapanax leschenaultii (DC.) Seem.

Polyscias acuminata (Wight) Seem.

Schefflera racemosa (Wight) Harms - (Kappa Maram, Kottathunikkan)

48. *Cornaceae*

Mastixia arborea (Wight) Bedd. - (Eramba Maram,Kunthirikkam - EBIS)

49. *Caprifoliaceae*

Viburnum coriaceum Blume - (Mottu Mookan - EBIS)

Viburnum erubescens Wall. *ex* DC.

Viburnum punctatum Buch.- Ham *ex* D. Don - (Konakkara - EBIS)

50. Rubiaceae

Canthium dicoccum (Gaertn.) Teijsm. & Binn., var. *umbellatum* (Wight) Sant. & Merch. – (Edali maram – EBIS)

Canthium neilgherrense Wight, var. *chartaceum* (Gamble) Swamin.

Canthium rheedii DC.

Chassalia curviflora (Wall.) Thw., var. *ophioxyloides* (Wall.) Deb & Krishna

Galium asperifolium Wall.

Hedyotis anamalayana (Gamble) Rao & Hemadri

Hedyotis articularis R. Br. *ex* Wight & Arn., subsp. *santapaui* (Shetty & Vivek.) Deb. & Dutta

Hedyotis leschenaultiana DC.

Hedyotis swertioides Hook. f. - (Thavalakkal Chedi)

Knoxia sumatrensis (Retz.) DC.

Lasianthus acuminatus Wight - (Peenari, Theettanari, Mootu Maram - EBIS)

Lasianthus coffeoides Fyson

Lasianthus venulosus (Wight & Arn.) Wight

Mussaenda hirsutissima (Hook. f.) Hutch. *ex* Gamble - (Chandrakala Sooryakala - EBIS)

Neanotis hutchinsonii Deb. & R. Dutta

*Neanotis indica (*DC.) W. H. Lewis, var. *affinis* (Wall. *ex* Wight & Arn.) W. H. Lewis

Neanotis indica (DC.) W. H. Lewis, var. *indica.*

Neanotis monosperma (Wall. *ex* Wight & Arn.) W. H. Lewis

Oldenlandia corymbosa L.

Ophiorrhiza brunonis Wight & Arn.

Ophiorrhiza grandiflora Wight

Ophiorrhiza roxburghiana Wight

Pavetta breviflora DC.

Psychotria glandulosa (Dennst.) Suresh in Nicolson

Psychotria nilgiriensis Deb & M. G. Gangop., var. *astephana* (Hook. f.) Deb. & M. G. Gangop. - (Pavadakkambu)

Psychotria nilgiriensis Deb & M.G. Gangop, var. *nilgiriensis* - (Kottappoovu, Pavadakkambu - EBIS)

Psychotria nudiflora Wight & Arn.

Rubia cordifolia L.

Saprosma foetens (Wight) K. Schum. - (*Theetanari* - EBIS)

Spermacoce ocymoides Burm. F.

Wendlandia thyrsoidea (Roemer & Schult.) Steud.

51. Valerianaceae

Valeriana hookeriana Wight & Arn.

Valeriana leschenaultii DC.

52. Asteraceae

Acanthospermum hispidum DC., Prodr. 5: 522. 1836 - (Musu Musu)

Acmella calva (DC.) R. K. Jansen - (Nai Koppu, Nai Manjal - EBIS)

Adenostemma lavenia (L.) O. Ktze.

Ageratina adenophora (Spreng.) King & Robins. - (Thravada, Nilagiri - EBIS)

Ageratum houstonianum Mill. - (Kaliyamman Pathiri Chedi, Michangala - EBIS)

Anaphalis aristata DC. - (Panji Poovu - EBIS)

Anaphalis lawii (Hook. f.) Gamble - (Panji Poovu - EBIS)

Anaphalis leptophyl la (DC) DC. - (Panji Poovu - EBIS)

Anaphalis marcescens (Wight) Clarke

Anaphalis meeboldii W.W. Sm. - (Panji Poovu - EBIS)

Anaphalis subdecurrens (DC.) Gamble - (Panji Poovu - EBIS)

Anaphalis travancorica W.W. Sm. - (Panji Poovu - EBIS)

Artemisia nilagirica (Clarke) Pamp. - (Velipathiri - EBIS)

Bidens pilosa L.

Cirsium wal lichii DC. - (Chakku Mullu) (EBIS).

Cissampelopsis corymbosa (Wall. *ex* DC.) C. Jeffrey & Y. L. Chen - (EBIS)

Cissampelopsis walkeri (Arn.) C. Jeffrey & Y. L. Chen - (Vettuva Marunnu Chedi – (EBIS)

Conyza stricta Willd.

Crassocephalum crepidioides (Benth) S. Moore

Dichrocephala chrysanthemifolia (Blume) DC.

Dichrocephala integrifolia (L. f.) O. Ktze.

Emilia scabra DC. - (Poosha Thala - EBIS)

Erigeron karvinskianus DC. - (Pottu Poovu - EBIS)

Galinsoga parviflora Cav.

Gamochaeta coarctata (Willd.) M. Kerguelen

Gamochaeta purpurea (L.) Cabrera

Gynura nitida DC.

Gynura travancorica Smith - (Moyalu Kola, Koppu Chedi - EBIS)

Helichrysum buddleioides DC. - (Panji Poovu - EBIS)

Kleinia grandiflora (DC.) N. Rani

Myriactis wightii DC.

Parthenium hysterophorus L.

Phyl locephalum scabridum (DC.) K. Kirkman

Senecio scandens Buch.-Ham. *ex* D. Don - (Pomburi Kodi - EBIS)

Sigesbeckia orientalis L.

Sonchus oleraceus L. - (Palkkola, Varapputhanal - EBIS)

Vernonia arborea Buch.-Ham. - (Shengurinji)

Vernonia bourneana W.W. Sm.

Vernonia fysonii Calder

Vernonia peninsularis (Clarke) Clarke *ex* Hook. f.

Vernonia saligna DC., var. *nilghirensis* Hook.f.

Youngia japonica (L.) DC.

53. *Campanulaceae*

Lobelia heyneana Roem. & Schult.

Lobelia leschenaultiana (Presl.) Skottsb. - (Thonnali - EBIS)

Lobelia nicotianiifolia Roth *ex* Schult.

Wahlenbergia erecta (Roth *ex* Schult.) Moeliono & Tuyn

Wahlenbergia marginata (Thunb.) A. DC.

54. *Ericaceae*

Gaultheria fragrantissima Wall. - (Kolgatte Chedi - EBIS)

Rhododendron arboreum Smith, subsp. *nilagiricum* (Zenk.) Tagg. - (Alangi - EBIS)

55. *Vacciniaceae*

Vaccinium leschenaultii Wight - (Kela Maram - EBIS)

Vaccinium neilgherrense Wight - (Kela Maram - EBIS)

56. *Primulaceae*

Lysimachia procumbens Baudo

57. *Myrsinaceae*

Ardisia rhomboidea Wight

Embelia ribes Burm. f.

Embelia tsjeriam-cottam (Roem. & Schult.) A. DC.

Maesa indica (Roxb.) A. DC. - (Kattu Malli, Vallara)

Myrsine thwaitesii (Mez) B. M. Wadhwa - (Cheera Maram - EBIS)

Myrsine wightiana Wall. *ex* DC. - (Cheera Maram - EBIS)

58. *Sapotaceae*

Isonandra lanceolata Wight - (Vella Pala - EBIS)

Isonandra perrottetiana A. DC. - (Karimbala - EBIS)

Xantolis tomentosa (Roxb.) Raf., var. *elengioides* (A. DC.) Vajr. - (Kara - EBIS)

59. *Symplocaceae*

Symplocos anamal layana Bedd.

Symplocos cochinchinensis (Lour.) Moore, subsp. *laurina* (Retz.) Nooteb. - (Kaatukappi, Parala - EBIS)

Symplocos foliosa Wight. - (*Parala* - EBIS)

Symplocos macrophyl la Wall. *ex* A. DC., subsp. *macrophyl la* Nooteb.

Symplocos macrophyl la Wall. *ex* A. DC., subsp. *rosea* (Bedd.) Nooteb.

Symplocos monantha Wight

Symplocos obtusa Wall. *ex* G. Don

Symplocos oligandra Bedd.

Symplocos pendula Wight

Symplocos pulchra Wight, var. *vil losa* (Brand.) Nooteb.

60. Oleaceae

Chionanthus linocieroides (Wight) Bennet & Raizada

Chionanthus ramiflorus Roxb., var. *peninsularis*, Ravikumar & Lakshmanan - (Kattu Chakkalathi, Vella Edali - EBIS)

Jasminum bignoniaceum Wall. *ex* DC.

Jasminum brevilobum. A.DC. - (Kattu Mulla - EBIS)

Ligustrum perrottetii DC. - (Pingan, Pingi - EBIS)

Ligustrum robustum (Roxb.) Blume, subsp. *walkeri* (Decne.) Green

Olea paniculata R.Br.

61. Apocynaceae

Alstonia venenata R. Br. – (Thipli, Thippili – EBIS)

Rauvolfia densiflora (Wall.) Benth. *ex* Hook. f.

62. Asclepiadaceae

Tylophora mol lissima Wight & Arn.

Wattakaka volubilis (L. f.) Stapf - (Kakklan Kodi - EBIS)

63. Loganiaceae

Fagraea ceylanica Thunb. - (Maruthanka Maram)

Gardneria ovata Wall.

64. Gentianaceae

Exacum wightianum Arn. - (Vella Thavalakkal Chedi - EBIS)

Gentiana pedicel lata (D. Don) Griseb., var. *wightii* Kusn.

Swertia corymbosa (Griseb.) Wight *ex* Clarke

Swertia lawii (Wight *ex* Clarke) Burkill

65. Boraginaceae

Cynoglossum zeylanicum (Vahl *ex* Hornem.) Thunb. *ex* Lehm

66. Convolvulaceae

Argyreia hirsuta Wight & Arn.

Cuscuta reflexa Roxb.

67. Solanaceae

Cyphomandra betacea (Cav.) Sendtn. - (Seemakkathiri, Mara Thakkali - EBIS)

Lycopersicon lycopersicum (L.) Karsten - (Chirangu Thakkali, Cheru Thakkali - EBIS)

Nicandra physalodes (L.) Gaertn. - (Oomathan)

Physalis peruviana L. - (Pottipalam, Perungunni, Malathakkali Keera - EBIS)

Solanum anguivi Lam., var. *anguivi*. - (Kandanka Mullu)

Solanum anguivi Lam., var. *multiflora* (Roth. *ex* Roem. & Schult.) Chitra - (Kandamka Mullu - EBIS)

Solanum giganteum Jacq.

Solanum nigrum L. - (Eeradakukkeera - EBIS)

Solanum virginianum L. - (Kandam Kathiri - EBIS)

68. Scrophulariaceae

Calceolaria gracilis Kunth. - (Nettipottu Chedi)

Dopatrium nudicaule (Willd.) Benth.

Lindernia ruel lioides (Colsm.) Pennell

Pedicularis zeylanica Benth.

Sopubia delphiniifolia (L.) G. Don

Striga asiatica (L.) O. Ktze.

69. Orobanchaceae

Aeginetia pedunculata (Roxb.) Wall.

Christisonia neilgherrica Gardner

Christisonia bicolor Gard.

Christisonia tubulosa (Wight) Benth. *ex* Hook. f.

70. Lentibulariaceae

Utricularia graminifolia Vahl

Utricularia striatula Sm.

71. Gesneriaceae

Aeschynanthus perrottetii A. DC.

Henckelia incana (Vahl) Spreng.

72. Acanthaceae

Andrographis neesiana Wight

Asystasia crispata Benth.

Barleria involucrata Nees, var. *elata* (Dalz.) Clarke

Justicia japonica Thunb.

Rungia laeta Clarke

Strobilanthes foliosus (Wight) T. Anders. - (Kallu-kurinji, Vettilakurinji - EBIS)

Strobilanthes gracilis Bedd. - (Thoka Kurinji - EBIS)

Strobilanthes homotropus Nees - (Thoka Kurinji)

Strobilanthes kunthianus (Nees) T. Anders. *ex*. Benth. - (Neelakkurunji - EBIS)

Strobilanthes luridus Wight - (Muttakannikurinji)

Strobilanthes micranthus Wight - (Kallankurinji)

Strobilanthes neilgherrensis Bedd.

Strobilanthes papillosus T. Anders.

Strobilanthes perrottetianus Nees - (Chonakurinji)

Strobilanthes pulneyensis Clarke - (Chonayam-kallu Kurinji)

Strobilanthes tristis (Wight) T. Anders.

Strobilanthes wightianus Nees

Strobilanthes zenkerianus (Nees) T. Anders

Thunbergia tomentosa Wall. *ex* Nees

73. Verbenaceae

Clerodendrum serratum (L.) Moon

Clerodendrum viscosum Vent. - (Kattu Padappa - EBIS)

Lantana camara L. - (Unni Mullu, Sankutheva Mullu - EBIS)

Lantana indica Roxb. - (Kuzhaloothi Palam - EBIS)

74. Lamiaceae (Lamiaceae)

Anisochilus argenteus Gamble - (Seethakkorali)

Anisomeles indica (L.) O. Ktze.

Clinopodium umbrosum (M. Bieb.) K. Koch

Isodon nilgherricus (Benth.) H. Hara

Leucas chinensis (Retz.) R. Br.

Leucas lamifolia Desf. - (Perum Thumba - EBIS)

Leucas lancifolia Desf.

Leucas rosmarinifolia Benth.

Leucas suffruticosa Benth.

Leucas ternifolia Desf.

Leucas vestita Benth. - (Hanuman Pal)

Leucas zeylanica (L.) R. Br.

Micromeria biflora (Buch.-Ham. *ex* D. Don.) Benth.

Plectranthus barbatus Andr.

Plectranthus coleoides Benth. - (Padappayila - EBIS)

Plectranthus malabaricus (Benth.) Willemse

Plectranthus mollis (Ait.) Spreng.

Pogostemon atropurpureus Benth. - (Kuzhaloothi Pazham - EBIS)

Pogostemon benghalensis (Burm. f.) O. Ktze.

Pogostemon pubescens Benth.

Pogostemon wightii Benth.

Prunella vulgaris L.

Scutellaria violacea Heyne *ex* Benth.

75. Plantaginaceae

Plantago erosa Wall - (Nilachakka, Njaramboori - EBIS)

76. Amaranthaceae

Achyranthes aspera L., var. *aspera* - (Nayaruvi Chedi)

Achyranthes aspera L., var. *pubescens* (Miq.) Townsend

Achyranthes bidentata Blume - (Nayaruvi Chedi)

Allmania nodiflora (L.) R. Br. *ex* Wight

Amaranthus viridis L. - (Arakkeera - EBIS)

Celosia pulchella Moq.

77. Chenopodiaceae

Chenopodium ambrosioides L. - (Natta Chedi - EBIS)

78. Phytolaccaceae

Phytolacca octandra L.

79. Polygonaceae

Persicaria chinensis (L.) Gross - (Mukkala, Oduthan - EBIS)

Persicaria glabra (Willd.) M. Gomez - (Cheru-mukkala)

Persicaria nepalensis (Meisner) Gross - (Cheru-mukkala)

Rumex nepalensis Spreng. - (Chethakkala - EBIS)

80. Podostemaceae

Polypleurum stylosum (Wight) Hall

81. Piperaceae

Peperomia portulacoides (Lam.) Dietr.

Peperomia tetraphylla (Forst.) Hook. & Arn.

Piper argyrophyllum Miq. - (Kattu Kurumulaku - EBIS)

Piper hymenophyllum Miq.

Piper mullesua Buch.-Ham. *ex* D. Don - (Kattu Thippali - EBIS)

Piper nigrum L. - (Kattu Kurumulaku, Eya Kodi - EBIS)

Piper schmidtii Hook. f. - (Kattu Kurumulaku, Eya Kodi - EBIS)

Piper trioicum Roxb. - (Kattu Kurumulaku - EBIS)

Piper wightii Miq. - (Kattu Kurumulaku, Eya - EBIS)

82. Lauraceae

Actinodaphne bourdillonii Gamble - (Vari Maram - EBIS).

Actinodaphne malabarica Balakr.

Apollonias arnottii Nees - (Chulunthu)

Beilschmiedia wightii (Nees) Benth. *ex* Hook. f. - (Panthada, Nagara Maram - EBIS)

Cinnamomum filipedicellatum Kosterm.

Cinnamomum keralaense Kosterm.

Cinnamomum macrocarpum Hook. f.

Cinnamomum malabatrum (Burm. f.) Blume - (Vella Kodala - EBIS)

Cinnamomum travancoricum Gamble

Cinnamomum wightii Meisner - (Vella Kodala, Santha Maram - EBIS)

Cryptocarya bourdillonii Gamble

Litsea coriacea (Heyne *ex* Meisner) Hook. f.

Litsea glabrata (Wall. *ex* Nees) Hook. f.

Litsea oleoides (Meisner) Hook. f. - (Chembalava, Shembalava - EBIS)

Litsea wightiana (Nees) Hook. f., var. *tomentosa* (Meisner) Gamble - (Manja Kodala - EBIS)

Litsea wightiana (Nees) Hook. f. - (Manja Kodala - EBIS)

Neolitsea cassia (L.) Kosterm. - (Mulaku Nari, Pravari - EBIS)

Neolitsea fischeri Gamble - (Vari Maram - EBIS)

Persea macrantha (Nees) Kosterm. - (Kulamavu - EBIS)

Phoebe wightii Meisner

83. Proteaceae

Grevillea robusta A. Cunn. *ex* R. Br. - (Silver Oak - EBIS)

84. Thymeleaceae

Gnidia glauca (Fresen.) Gilg. - (Nachu Nar, Nanju Nar - EBIS)

85. Elaeagnaceae

Elaeagnus indica Servett.

Elaeagnus kologa Schlecht. - (Poolam Palam - EBIS)

86. Loranthaceae

Helixanthera intermedia (Wight) Danser - (Puluri)

Helixanthera obtusata (Schult.) Danser

Macrosolen capitellatus (Wight & Arn.) Danser

Macrosolen parasiticus (L.) Danser, Blumea 2: 36. 1936

Taxillus cuneatus (Heyne *ex* Roth) Danser - (Ottu Puluri)

Taxillus tomentosus (Heyne *ex* Roth) Tieghem

87. Viscaceae

Korthalsella japonica (Thunb.) Engl.

Viscum ramosissimum Wall. *ex* Wight & Arn. - (Mara Theva)

88. Balanophoraceae

Balanophora dioica R.Br.

Balanophora fungosa J. R. & G. Forst., subsp. *indica* (Arn.) Hansen, var. *indica* - (Kannukutti Madu - EBIS)

89. Euphorbiaceae

Antidesma menasu (Tul.) Miq. *ex* Muell.-Arg.

Bischofia javanica Blume - (Thondi, Chorakkali - EBIS).

Breynia retusa (Dennst.) Alston - (Aattacherukola - EBIS).

Daphniphyllum neilgherrense (Wight) K. Rosenth. - (Vellakotlan, Peekkiri, Kozhikulamavu)

Euphorbia rothiana Spreng. - (Merasupal Chedi - EBIS)

Excoecaria crenulata Wight

Glochidion candolleanum (Wight & Arn.) Chakrab. & Gangop. - (Chendana, Chathakkadambu - EBIS)

Glochidion ellipticum Wight - (Chendana, Chathakkadambu - EBIS)

Macaranga indica Wight - (Vattakanni - EBIS)

Mallotus philippensis (Lam.) Muell.-Arg. - (Thakiri)

Mallotus tetracoccus (Roxb.) Kurz. - (Vattakanni, Perakela - EBIS)

Phyllanthus macraei Muell.-Arg.

Phyllanthus rheedii Wight

Ricinus communis L. - (Amanakku, Kotta Maram - EBIS)

90. Buxaceae

Sarcococca saligna (D. Don) Muell.-Arg. - (Mattu Vadi - EBIS)

91. Urticaceae

Boehmeria macrophylla Hornem.

Debregeasia longifolia (Burm. f.) Wedd. - (Neeranji, Narambili, Vanchi - EBIS)

Elatostema lineolatum Wight, var. *falcigera* Thw.

Elatostema sessile J.R. Forst. & G. Forst.

Girardinia diversifolia (Link) Friis - (Ana Chenthatti - EBIS)

Laportea terminalis Wight - (Cheriya Chenthatti)

Pilea kingii Fischer

Pilea melastomoides (Poir.) Wedd. - (*Narali* - EBIS)

Pilea wightii Wedd. - (Valiya Narali - EBIS)

Pouzolzia auriculata Wight

Pouzolzia bennettiana Wight, var. *acuta* (Wight) Fischer - (Narali Kola, Serathandan - EBIS)

Pouzolzia wightii Benn. - (Narali Kola - EBIS)

Procris crenata Rob.

92. Ulmaceae

Celtis philippensis Blanco, var. *wightii* (Planch.) Soep. - (Vellakkuyyan - EBIS)

Celtis tetrandra Roxb. - (Karukkuyyan, Sarala Kuyyan - EBIS)

Trema orientalis (L.) Blume - (Oma Maram - EBIS)

93. Cannabaceae

Cannabis sativa L. - (*Kanchavu* - EBIS)

94. Moraceae

Dorstenia indica Wall. *ex* Wight

Ficus laevis Blume, var. *macrocarpa* (Miq.) Corner - (Peyathi)

95. Salicaceae

Salix tetrasperma Roxb. - (Vanchi Maram - EBIS)

II. Angiosperms: Monocotyledons

96. Orchidaceae

Aerides ringens (Lindl.) Fischer

Anoectochilus elatus Lindl.

Brachycorythis iantha (Wight) Summerh.

Brachycorythis splendida Summerh.

Calanthe sylvatica (Thouars) Lindl.

Calanthe triplicata (Willem.) Ames

Cheirostylis flabellata Wight

Chrysoglossum maculatum (Thwaites) Hook. f.

Cirrhopetalum gamblei Hook. f.

Dendrobium anamalayanum Chandr.

Dendrobium nanum Hook. f.

Diplocentrum recurvum Lindl.

Eria dalzellii Lindl.

Eria pauciflora Wight

Habenaria longicornu Lindl.

Habenaria perrottetiana A. Rich.

Liparis walkerae Grah.

Liparis wightiana Thw.

Oberonia chandrasekharanii Nair *et al.*

Oberonia thwaitesii Hook. f.

Oberonia verticillata Wight

Oberonia wightiana Lindl.

Satyrium nepalense D. Don

Seidenfadeniella filiformis (Reichb. f.) Chr. & Ormerod.

Seidenfia rheedii (Sw.) Szlach.

Taeniophyllum alwisii Lindl.

Trichoglottis tenera (Lindl.) Schltr.

97. Zingiberaceae

Alpinia abundiflora Burtt & Smith - (Kattu Elam)

Hedychium flavescens Carey *ex* Rosc. - (Navakkutti, Kooku Vala - EBIS)

98. Haemodoraceae

Ophiopogon intermedius D. Don

Peliosanthes courtallensis Wight

99. Iridaceae

Aristea ecklonii Baker

100. Amaryllidaceae

Crinum latifolium L.

101. Hypoxidaceae

Curculigo orchioides Gaertn. - (Nilaippana - EBIS)

Molineria trichocarpa (Wight) Balakr.

102. Liliaceae

Chlorophytum attenuatum (Wight) Baker

Disporum leschenaultianum D. Don

Lilium neilgherrense Wight - (Thatha Poovu, Thazhamboo)

Protasparagus fysonii (Macbr.) Kamble

Protasparagus racemosus (Willd.) Oberm. - (Pilichaviri, Thaali Periyan - EBIS)

103. Smilacaceae

Smilax aspera L. - (Pomburi Kodi)

Smilax wightii A. DC. - (Choora Mullu)

104. Xyridaceae

Xyris capensis Thunb., var. *schoenoides* (Mart.) Nilsson

105. Commelinaceae

Commelina clavata Clarke - (Kalani)

Commelina paleata Hassk.

Cyanotis arachnoidea Clarke

Cyanotis cristata (L.) D. Don

Cyanotis fasciculata (Heyne *ex* Roth) Schult. f.

Cyanotis pilosa Schult. f. - (Kalani - EBIS)

Cyanotis villosa (Spreng.) Schult. f.

106. Juncaceae

Juncus bufonius L.

Juncus effusus L.

Juncus inflexus L.

Juncus leschenaultii Gay

107. Arecaceae (Palmae)

Calamus gamblei Becc. *ex* Becc. & Hook. f. - (Chooral - EBIS)

108. Araceae (Aroideae)

Arisaema attenuatum Barnes & Fischer - (Pambu Cholam)

Arisaema barnesii Fischer

Arisaema leschenaultii Blume - (Pambu Cholam)

Arisaema murrayi (Graham) Hook. - (Pambu Cholam)

Arisaema psittacus Barnes in Hook. - (Pambu Cholam)

109. Eriocaulaceae

Eriocaulon brownianum Mart. *ex* Wall. - (Valiya Button Poovu - EBIS)

Eriocaulon collinum Hook. f. - (Button Poovu - EBIS)

Eriocaulon melaleucum Mart. - (Button Poovu - EBIS)

Eriocaulon odoratum Dalz. - (Button Poovu - EBIS)

110. Cyperaceae

Carex baccans Nees *ex* Wight - (Cheru Korappullu)

Carex brunnea Thunb.

Carex filicina Nees

Carex lindleyana Nees, var. *lindleyana* Clarke - (Korappullu)

Carex lindleyana Nees, var. *major* (Steud.) Fischer

Carex longicruris Nees

Carex myosurus Nees

Carex nubigena D.Don

Carex phacota Spreng.

Carex raphidocarpa Nees

Cyperus compressus L.

Eleocharis congesta D. Don,

Fimbristylis dichotoma (L.) Vahl., var. *villosa* Fischer

Fimbristylis kingii Gamble *ex* Boeck.

Fimbristylis uliginosa Hochst. *ex* Steud.

Kyllinga nemoralis (J. R. Forst. & G. Forst.) Dandy *ex* Hutch. & Dalz.

Mariscus paniceus (Rottb.) Vahl

Pycreus flavidus (Retz.) Koyama

Schoenoplectus mucronatus (L.) Palla

111. Poaceae

Apluda mutica L.

Arundinaria densifolia Munro

Arundinaria floribunda Thw. - (Eeta - EBIS)

Arundinella vaginata Bor - (Chonappullu)

Chrysopogon nodulibarbis (Steud.) Henrard

Cymbopogon flexuosus (Nees *ex* Steud.) Wats. - (Inchipullu, Teruvapullu, Vevvapullu - EBIS)

Cymbopogon travancorensis Bor - (Kattuvevvapullu)

Eragrostis nigra Nees *ex* Steud.

Eragrostis tef (Zucc.) Trotter

Eragrostis tenuifolia (A. Rich.) Hochst. *ex* Steud.

Eragrostis unioloides (Retz.) Nees *ex* Steud. - (Karayampullu)

Eulalia phaeothrix (Hack.) O. Ktze.

Garnotia tenella (Arn. *ex* Miq.) Janowski

Helictotrichon virescens (Nees *ex* Steud.) Henr.

Isachne bourneorum Fischer

Isachne walkeri (Arn. *ex* Steud.) Wight & Arn. *ex* Thw.

Ischaemum indicum (Houtt.) Merr., var. *indicum* subvar. *villosum* (Nees) Bor

Microstegium ciliatum (Trin.) A. Camus

Microstegium nudum (Trin.) A. Camus

Oplismenus burmannii (Retz.) P. Beauv.

Oplismenus compositus (L.) P. Beauv.

Panicum gardneri Thw. - (*Kolapullu*)

Poa annua L.

Pseudoxytenanthera monadelpha (Thw.) Soderstrom & Ellis - (Kaattu Mula - EBIS)

Rhynchelytrum repens (Willd.) C.E. Hubb.

Setaria paniculifera (Steud.) Fourn. *ex* Hemsl. - (Nayurippullu)

Setaria pumila (Poir.) Roem. & Schult. - (Kambilipullu)

Sorghum bicolor (L.) Moench.

Sorghum nitidum (Vahl) Pers.

Themeda triandra Forssk. - (Chekkanampullu)

Tripogon bromoides Roem. & Schult.

Zenkeria elegans Trin.

III. Pteridophytes

1. Lycopodiaceae

Huperzia hilliana (Nessel) Holub

Huperzia macrostachys (Hook. *ex* Spring) Holub

Huperzia phlegmaria Rothm.

Lycopodiella cernua (L.) Pic. Serm.

Lycopodium japonicum Thunb.

Lycopodium wightianum Wall. *ex* Hook. & Grev.

2. *Selaginellaceae*

Selaginella brachystachya (Hook. & Grev.) Spring - (Urakkenni Poovu - EBIS)

Selaginella tenera (Hook. & Grev.) Spring

Selaginella involvens (Swarts) Spring

3. Psilotaceae

Psilotum nudum (L.) P. Beauv

4. Ophioglossaceae

Botrychium daucifolium Wall. *ex* Hook. & Grev.

5. Marattiaceae

Marattia fraxinea Sw.

6. *Pteridaceae*

Pteris argyrea T. Moore

Pteris confusa T. G. Walker

Pteris cretica L.

Pteris quadriaurita Retz. - (Njandu Thuravan - EBIS)

7. Sinopteridaceae

Cheilanthes farinosa (Forsk.) Kaulf.

Cheilanthes thwaitesii Mett.

Doryopteris concolor (Langsd. & Fischer) Kuhn

Pellaea falcata Bedd.

8. Hemionitidaceae

Parahemionitis cordata (Roxb. *ex* Hook. & Grev.) Fraser-Jenk. - (Pattichevi, Poonakkathi - EBIS)

Pityrogramma austro-americana Domin

9. Adiantaceae

Adiantum raddianum C. Presl

10. Vittariaceae

Antrophyum plantagineum (Cav.) Kaulf.

Vittaria elongata Sw.

11. Dennstaedtiaceae

Microlepia speluncae (L.) T. Moore

Pteridium aquilinum (L.) Kuhn - (Chulliveru Chedi)

12. Lindsaeaceae

Odontosoria chinensis (L.) J. Sm.

13. Davalliaceae

Araiostegia pulchra (D. Don) Copel

14. Oleandraceae

Nephrolepis auriculata (L.) Trimen

15. Hymenophyllaceae

Crepidomanes plicatum (Bosch.) R. C. Ching

16. Gleicheniaceae

Dicranopteris linearis var. *sebastiana* Panigrahi & Dixit

17. Cyatheaceae

Alsophila nilgirensis (Holttum) R. M. Tryon - (Pana Edaval, Kalyana Thevai - EBIS)

Sphaeropteris crinita (Hook.) R. M. Tryon - (Pana Edaval, Kalyana Thevai - EBIS)

18. Thelypteridaceae

Pseudocyclosorus ochthodes (Kunze) Holttum var. *annamalayensis* Manickam & Irudayaraj

Pseudocyclosorus ochthodes (Kunze) Holttum var. *palniensis* Manickam & Irudayaraj

Pseudocyclosorus tylodes (Kunze) Ching

Pseudophegopteris pyrrhorhachis (Kunze) Ching

Stegnogramma pozoi (Lagasca) K. Iwats

Thelypteris dentata (Forssk.) E. P. St. John

19. Aspleniaceae

Asplenium aethiopicum (Burm. f.) Becherer

Asplenium auritum Sw.

Asplenium decrescens Kunze

Asplenium erectum Bory *ex* Willd.

Asplenium formosum Willd.

Asplenium inaequilaterale Willd.

Asplenium normale D. Don

Asplenium polyodon G. Forst.

Asplenium tenuifolium D. Don

Asplenium unilaterale Lam.

Asplenium zenkeranum Kunze

Asplenium sp.

20. Athyriaceae

Athyrium solenopteris (Kunze) T. Moore

Deparia petersenii (Kunze) M. Kato

Diplazium dialatatum Bl.

Diplazium esculentum (Retz.) Sw.

Dryoathyrium boryanum (Willd.) Ching

21. Dryopteridaceae

Arachniodes aristata (G. Forst.) Tindale

Arachniodes tripinnata (Goldm.) Sledge

Dryopteris cochleata (D.Don) C.Chr

Dryopteris hirtipes (Blume) Kuntze

Dryopteris sparsa (Buch. - Ham. *ex* D. Don) Kuntze

Polystichum harpophyllum (Zenker *ex* Kunze) Sledge

Polystichum piceopalaceum Tagawa

22. Lomariopsidaceae

Elaphoglossum stelligerum (Wall. *ex* Baker) T. Moore *ex* Alston & Bonner

23. Polypodiaceae

Crypsinus montanus Sledge

Lepisorus amaurolepidus (Sledge) Bir & Trikha

Lepisorus nudus (Hook.) Ching

Leptochilus axillaris (Cav.) Kaulf.

Leptochilus decurrens Blume

Leptochilus decurrens Blume *forma lanceolatus*

Loxogramme cuspidata (Zenker) M. G. Price

Pleopeltis macrocarpa (Bory *ex* Willd.) Kaulf

Pyrrosia porosa Hovenkamp

24. Grammitidaceae

Ctenopteris perplexa Parris

Grammitis attenuata Kunze

Grammitis sp.

During this study, about 674 taxa of vascular plants, which include 598 taxa of flowering plants and 76 pterdidophytes, could be collected from the park. The 598 taxa of flowering plants belonged to 356 genera under 111 families. Dicotyledons were represented by 484 taxa belonging to 288 genera under 95 families. Monocotyledons were represented by 114 taxa in 68 genera under 16 families. In the case of pteridophytes, the 77 taxa belonged to 46 genera under 24 families.

Among the dicots, *Asteraceae* with 42 taxa belonging to 28 genera was the largest family. It was followed by *Fabaceae* (41/19), *Rubiaceae* (35/16),

Lamiaceae (22/10), *Acanthaceae* (19/7) etc. Among the monocots, *Poaceae* with 32 taxa under 22 genera was the largest family. It was followed by *Orchidaceae* (27/18), *Cyperaceae* (18/8), *Commelinaceae* (7/2), *Araceae* (5/1) etc.

Among the pteridophytes, *Aspleniaceae* with 12 taxa under 1 genus was the largest. It was followed by *Polypodiaceae* (9/6), *Dryopteridaceae* (7/3), *Thelypteridaceae* (6/4), *Lycopodiaceae* (6/3), *Athyriaceae* (5/4) etc.

Habit and Habitat

Regarding the habit of the plants, out of the 484 dicot taxa, 191 (39.5%) belonged to herbs, 163 (33.7%) were shrubs 130 (26.9%) were trees. Out of the 219 herbs, 20 were climbers and 10 were parasitic. Climbers include species of *Tylophora, Diplocyclos, Solena, Trichosanthes, Zehneria, Cajanus, Canavalia, Desmodium, Dumasia, Rhynchosia, Shuteria, Vigna, Clematis,* etc. Parasitic herbs include various species of *Balanophora, Utricularia, Christisonia, Striga asiatica,* etc. The lianas reported include *Cayratia pedata, Cissampelopsis corymbosa, C. walkeri, Connarus wightii, Derris brevipes, Ficus amplocarpa* and *Parthenocissus semicordata*. Parasitic shrubs mainly belong to *Loranthaceae* and *Viscaceae* (*Helixanthera, Macrosolen, Taxillus, Korthalsella, Viscum,* etc). Among the 114 monocot taxa, 108 (94.7%) belonged to herbs. Shrubby monocots include reed bamboo species such as *Arundinaria densifolia* and *A. floribunda* other than *Pseudoxytenanthera monadelpha, Protasparagus fysonii, P. racemosa,* etc.

Among the 76 pteridophytes, terrestrial taxa were more (42) than the epiphytes, and lithophytes. Seven taxa were either terrestrial or lithophytic. Similarly plants, which grow both as epiphytic and lithophytic, constituted 12 taxa. The genus *Asplenium* was distributed in all the above-mentioned groups. Family members of *Grammitidaceae, Lomariopsidaceae, Hymenophyllaceae, Vittariaceae,* etc. were epiphytic. Similarly, *Athyriaceae, Dryopteridaceae, Pteridaceae* and *Thelypteridaceae* members were terrestrial. The tree fern species include *Alsophila nilgirensis* and *Sphaeropteris crinita,* which were formerly included under the genus *Cyathea*. The pteridophytes constitute a major part of the shola vegetation.

Distribution of the species

It became evident that distribution of more than 30 percent of the plants collected during this study was restricted to Western Ghats. About 16 percent of plants are common to South India and Srilanka. Indo-Malesian species constitute about 8 percentage. About 3 percentage of plants are of Southeast Asian distribution. Out of the total number of 598 angiosperm taxa, nearly 5% are exotics. Some of these are introduced ornamentals escaped from the gardens or mere weeds (*Acanthospermum hispidum, Ageratina adenophora, Ageratum houstonianum, Calceolaria gracilis, Desmodium uncinatum, Erigeron karvinskianus, Galinsoga parviflora, Gamochaeata coarctata, Lantana camara, Oenothera laciniata, Parthenium hysterophorus, Chenopodium ambrosioides* etc). Since the park was bordered by several human settlements, certain exotic cultivated species have migrated into the wild, especially in the low altitude regions. This include vegetables or fruits such as *Cyphomandra betacea, Lycopersicon lycopersicum, Passiflora edulis, P. ligularis, P. mollissima,* etc. The introduction of exotic fodder grasses by the Livestock Development Board has resulted in the influx of the seeds of several other herbaceous plants also, which have now naturalised in the wild. Some introduced and widely cultivated trees have also entered into sholas (*Acacia dealbata, A. mearnsii, A. dealbata, Eucalyptus globulus, E. grandis, Grevillea robusta* etc). The role of birds, animals and man in the dispersal of the seeds of these species was noteworthy.

Endemism

The importance of shola forests which are considered, as the megacentres of endemism (Basha & Nair[87] & Nair & Basha[88]) becomes evident from the present study also. Out of the total 598 angiosperm taxa, 222 (37%) were endemics. This ratio was very huge, when compared with the scenario of the country (only 33.5%). Among the pteridophytes, out of the 76 taxa, only 4 taxa (5.2%) were found to be endemic. This indicates that pteridophytes comparatively have a wide range of (often cosmopolitan) distribution.

The Peninsular Indian endemic genera (6 nos.) reported from the park includes *Campbellia, Diplocentrum, Helicanthes, Nilgirianthus, Phlebophyllum* and *Vanasushava*. Among these, *Nilgirianthus* and *Phlebophyllum* are now treated under *Strobilanthes*. Genera such as *Arisaema, Hedyotis, Impatiens, Strobilanthes, Ophiorrhiza* and *Vernonia* are represented by more than 3 endemic species in Kerala, many of which were reported from the shola forests only. Among the dicots, majority of the South Indian

endemics (6 taxa) belonged to the family *Lamiaceae,* while *Asteraceae* (7 taxa) and *Rubiaceae* (21 taxa) had the highest number taxa as Western Ghat and Southern Western Ghat endemics. Out of the total number of 23 *Impatiens* reported, 18 (73 %) were endemics (14 species were Southern Western Ghat endemics and 4 were Western Ghat endemics). Similarly, out of the total number of 15 *Strobilanthes* reported, 13 (86.7%) were endemics (Twelve were Western Ghat endemics and one was South Indian endemic). Among the *Rubiaceae,* all the species of *Hedyotis* (4),*Lasianthus* (3) and *Psychotria* (3) were S. W. Ghat endemics. Same was the case with all the species of *Actinodaphne* (2) and *Cinnamomum* (6) of *Lauraceae*.

In the case of monocots, out of the 27 *Orchidaceae* members, 8 (29.6%) were S.W. Ghats endemics and 3 were South Indian endemics. Among the five *Arisaema* (*Araceae*) collected, 4 were S. W. Ghats endemics and one is W. Ghats endemic.

Among the pteridophytes, *Psuedocyclosorus, Sphaeropteris* (earlier name *Cyathea*) and *Gleichenia* coming under *Thelypteridaceae, Cyatheaceae* and *Gleicheniaceae* were the three genera, which have representation of endemic taxa.

Plants under different threat categories

Joseph[89], Henry *et al*[90], Jain and Sastry[91], Ahmedullah and Nayar[92] and Nayar and Sastry[93] have well documented the rare and threatened plants of South India. Nayar[94] had listed out 1272 endemic taxa in Kerala and 460 of them were placed under threat categories. Since sholas are centers of endemism, with more than one third (37 %) of the taxa being endemic as evidenced from the present study, chances of local extinction of species is far great. The habitat destruction and other anthropological activities have speeded up this process. So the figures of rare and threatened plants as documented in the above works are always not dependable, since majority of these works are based on secondary data or those based on the earlier herbarium collections. Thorough field investigation studies are necessary to validate many of this information. In general, 128 species (25%) classified as '*rare*' and another 26 species which were considered to be in a '*threatened state*' were reported from the park.

It is a fact that a lot of other species, which were not reported in the works cited earlier, were found locally in very rare and threatened conditions. Since sholas are facing several threats, documentation of such information deserves attention in the conservation point of view. Because many of such locally endangered taxa may face extinction, if proper conservation measures are not taken up.

Rediscovery of plants categorized as

'*Possibly extinct*'

During the study four species of angiosperms, earlier documented as '*Possibly extinct*' could be rediscovered which is given in Table 1 (also see Kishore kumar and Sasidharan[63,64]).

Arisaema attenuatum and *A. psittacus* were treated as '*possibly extinct*' by Shetty and Vivekananthan[22]. *A. attenuatum* could be collected from the Chengalar dam region at Mannavan Shola. This species is occasionally found in the shola borders or scrublands. *A. psittacus* is a threatened, narrow endemic species which could not be relocated after the type collection by Barnes in 1937 from Chunduvurrai & Mannavan Shola. It was so considered extinct in its natural habitat. However, the species was also rediscovered from the same localities by Nair *et al.*[28]. The species could be collected from both these locations and also from another National Park named Pambadam Shola, which is situated nearby.

Pimpinel la pulneyensis was so far reported from it's type locality (Palni hills) only and was

Table 1. 'Possibly extinct'* plants rediscovered during the study

No.	Name of the species	Habit	Family	References
1.	*Arisaema attenuatum* Barnes & C.E.C. Fisch.	Herb	Araceae	Shetty & Vivekananthan[22]; Nair & Basha[88]
2.	*Arisaema psittacus* Barnes	Herb	Araceae	Nair *et al.*[28]
3.	*Pimpinel la pulneyensis* Gamble	Herb	Apiaceae	Nayar[94]; Matthew[11]
4.	*Symplocos monantha* Wight	Tree	Symplocaceae	Nayar[94]

considered '*Possibly extinct*' (Nayar[94]; Matthew[11]). Present collection from Idlimottai region of Mannavan Shola thus forms both a rediscovery and extension of distribution of the species to Kerala. *Symplocos monantha* was also considered extinct by Nayar[94] since it could not be relocated after the type collection, which was made from the Shevagherry Hills of Tamil Nadu by Robert Wight[1], until it was reported from Periyar Tiger Reserve (Sasidharan[95]). The present collection from the Idlimottai regions of Mannavan Shola extends the distribution of the species.

New records

About 66 plants were reported for the first time from the State of Kerala as a result of this study. This include 44 angiosperms and 12 pteridophytes. These figures form about 7.4% of the total angiosperms collected (598 taxa) and 15.8% of the total pteridophytes collected (76 taxa), which is a substantially high figure. Among these, two were rediscoveries of plants considered as '*possibly extinct*' (*Pimpinel la pulneyensis* and *Symplocos monantha*), while one was a new record to the country (*Oenothera laciniata* – Onagraceae). Among these newly recorded plants, 12 species were ethnobotanically important also.

Details regarding the same are available in Kishore kumar[50,96], Kishore kumar and Muktesh kumar[49], Kishore kumar *et al*[56], Rajesh and Kishore kumar[65], Kishore kumar and Sasidharan[62-64] etc.

Diversity of Pteridophytes

The total number of 76 pteridophytes collected from park forms 23% of the total pteridophyte flora of the state (331 species: Easa[97]). The significance of this figure becomes clear when we compare the small area of park (33.45 km^2) to the total area of the Kerala state as a whole (38,863 km^2). It is quite interesting to see that ANSP which form only less than 0.086 percentage of the total forested land of Kerala is supporting 23% of the pteridophytes of the state.

Very rare and interesting tree ferns, which were considered as '*fossil plants*', are found to grow luxuriently in this park, where they are facing several threats of destruction (Kishore[51]). The need for conserving the such shola forest regions, which acts as a refugium of pteridophyte diversity, becomes evident here. Since shola forests in general harbour most remote and hostile mountainous tracts having diverse microclimates, thorough investigations will certainly yield several other taxa new to the world.

Conclusions

From the study it became clear that Anamudi Shola National Park is one of the most diverse ecosystems in the Western Ghats, which is considered as one of the 'hottest' of the hotspots of biodiversity. The total of 598 angiosperm taxa collected from a small area certainly indicates the high diversity prevailing in these regions. The 76 taxa of pteridophytes which contribute about 23% of the total flora of the state, collected from such a small area also signifies the high pteridophyte diversity of the region. Out of the total of 598 angiosperm taxa collected, 222 (37%) are endemics. These figures substantiates the general view that sholas as megacentres of endemism. Perhaps the unique altitudinal and climatic factors, which favour diverse microclimatic situations, might be leading to the high endemic concentration and diversity in these regions.

A number of plants belonging to various threat categories could be located by this study. Four plants, which were considered as 'possibly extinct', could also be rediscovered. Some 66 plants which includes 44 angiosperms and 12 pteridophytes collected from the park was reported for the first time from Kerala. All these studies highlights the importance of this park as a refugia of several endemic, rare and endangered taxa. It also highlights the need for conservation of the park, which faces several anthropogenic threats of destruction. Thorough explorations in the area may also lead to the discovery of more new taxa, since some of the regions remain still unexplored due to inaccessibility. The study also points out the need for conserving other Shola forests regions which are floristically and ecologically unique in all respects, before they get destroyed due to degradation, depletion or conversion of natural vegetation.

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Bioefficacy of selected botanicals in the management of rice weevil, *Sitophilus Oryzae* (L.) (Coleoptera: Curculionidae)

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Abstract

The rice weevil, *Sitophilus oryzae* is one of the major pests of stored grains. This study investigates the bio efficacy of selected botanicals-*Glycosmis pentaphyl la* and *Acanthus ilicifolius* in controlling infestations of the rice weevil. Laboratory experiments were conducted to study the mortality rate of the five doses (1%, 5%, 10%, 15%, and 20%) of acetone, ethanol and aqueous extracts of selected plants on the newly emerged adult *Sitophilus oryzae.* The efficacy of the extracts on the test insect was dose dependent. In this study acetone leaf extract of *Glycosmis pentaphyl la* produce higher mortality rate against *Sitophilus oryzae*. *Glycosmis pentaphyl la* thus proved to have strong pesticidal activity against *Sitophilus oryzae*, So it could be integrated into pest management system.

Key word: *Sitophilus oryzae*, *Glycosmis pentaphyl la*, *Acanthus ilicifolius*, insecticidal activity, mortality rate.

Introduction

In India, unscientific storage, rodents, insect pests, mites, microorganisms account for 10 per cent wastage of food grains. In comparison to other regions of the country, storage of food grains in North East India is very difficult due to high humidity, encouraging infestation of stored grains. Injudicious uses of chemicals as a pest management method have lead to the development of pesticide resistance in insects and create high degree of residual effect due to their non-degradable nature[3]. In Indo-Pak region, farmers have inherited knowledge of mixing leaves, barks, seeds, roots and oils of traditional plants with the stored grains for protection against insect pests[10.] Research reveals that extracts prepared from plants have a variety of properties including insecticidal activity, repellence to pests, anti feedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens[9.]

Botanical insecticides are considered as an alternative to synthetic chemicals for being biodegradable, pest specific, non-hazardous to human health as well as environment and leaving non toxic residues in nature[4]. Keeping this in view, the present study was carried out to test the pesticidal potential of the plant leaves *Glycosmis pentaphyl la* and *Acanthus ilicifolius* against the rice weevil, *Sitophilus oryzae*

Glycosmis pentaphyl la is an evergreen medicinal herb widely present in India with aromatic leaves used in the treatment of cough, rheumatism, anaemia, jaundice and ascariasis[5] fever and liver complaints, eczema and skin affections[8].

Acanthus ilicifolius, commonly known as Holly-leaved Acanthus, Sea Holly, and Holy Mangrove is a small shrub growing along lakes and marshes and sea shores. The plant is used for dyspepsia, paralysis, and asthma. The leaves are reported to be used in headache, rheumatism, and in skin diseases. Leaves and shoots are used as antidote in snake bite.

Considering their medicinal value and hoping that these test plants may yield environmentally sound chemicals and have no harmful effects on

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the non target organisms. The present study aims to focus on its effect on rice weevil, one of the most serious stored grain pests.

Materials and Methods

Culturing of test insects

The pest, *Sitophilus oryzae,* was collected from stored rice from a local shop. Fresh rice was washed and dried in sunlight. This rice was taken in containers and the insects were transferred to it. Thus stock cultures were prepared. Holes were drilled on the container lid for permitting the passage of air. The culture was maintained at room temperature. For getting newly emerged adults, 100 insects, including both male and female, from the stock were transferred to fresh rice. They were allowed to lay eggs on fresh rice. Then after 2 weeks, they were removed, and the rice containers were kept undisturbed. On the sixth week, new insects began to emerge in the containers. These insects were used for further studies. The stock culture was cleaned by sieving once in five days. This helps to remove the food waste and faecal matter of the insects to avoid fungal attack.

Preparation of extraction

Fresh leaves of the selected plants namely *Glycosmis pentaphyl la* and *Acanthus ilicifolius* were collected and shade-dried. The powdered plant materials were serially extracted using solvents in the increasing order of their polarity, i.e, acetone, ethanol and water by occasional warming and shaking. For these 50 grams of leaf powder were separately mixed with 250ml of solvents and stirred for 30 minutes and then left to stand for 24 hours. The mixture was then filtered through Whatman no.1 filter paper. The solvent from the filtered solution was evaporated in water bath. After complete evaporation of solvents, the final extract weighed and preserved in sealed bottles in a refrigerator until used for insect bioassays

Treatments

The extracts were applied at different doses on Whatmann No. 1 filter paper and air-dried for an hour. For this 0.2g, 1g, 2g, 3g and 4g (1%, 5%, 10%, 15%, and 20%) extracts were weighed separately and dissolved in 1 ml of distilled water separately. The controls were treated with acetone or ethanol or distilled water only. The treated and control filter paper discs were placed singly at the bottom of plastic jars and 20gm rice were placed on the papers. Ten insects *were* released in each plastic container. There were five replicates for each treatment and control. Observations were recorded on the seventh day of treatment.

Bioassay

All tests were conducted at optimal laboratory conditions. The observation of mortality was made on the seventh day of the study. The percentage of mortality was calculated by using the formula as given below.

Percentage of mortality (%) = (Number of dead insects x 100) / Number of insects introduced.

The data obtained are recorded as mean± standard deviation.

Table 1. Effect of extract on adult insects of *Sitophilus oryzae*

Dose (%)	Mortality rate (%)					
	Glycomis pentaphylla			Acanthus ilicifolius		
	Acetone	Ethanol	Aqueous	Acetone	Ethanol	Aqueous
1%	70±0.40	50±0.44	30±0.40	00±0.00	00±0.00	00±0.00
5%	80±0.09	70 ±0.09	40±0.20	00±0.00	00±0.00	00±0.00
10%	86±0.12	75±0.14	50±0.45	10±0.44	10±0.44	10±0.44
15%	86±0.20	78±0.20	60±0.45	20±0.54	10±0.54	10±0.54
20%	88±0.45	80±0.09	70±0.40	20±0.54	20±0.54	20±0.54

Note: Each datum represents the mean ± S.D. of five replicates (n=5).

Fig.1. Effect of different extract of *Glycosmis pentaphylla* on adult insects of *Sitophilus oryzae*.

Results and Discussion

The percentage of mortality rate on the adult insects of *Sitophilus oryzae* at different doses (1%, 5%, 10%, 15% and 20%) of acetone, ethanol and aqueous extracts were summarised in Table 1. No mortality was observed in the control.

The use of plant extracts to control stored products insects is an ancient practice. Insecticidal properties of a number of plant extract have been evaluated against stored product insects[1].In the present study significant mortality of the insect pest was observed in a dose dependent manner Reports show that deterrent effects of plant compounds are concentration dependant and may lead to complete mortality at high concentration[7]. The uses of indigenous plant products and locally available materials to protect stored cereals and legumes have been reported by many workers [6].

Results obtained in the present investigation clearly demonstrate that both solvent and aqueous extracts of *Glycosmis pentaphyl la* is toxic to *Sitophilus oryzae.* In this study application of the acetone extract of *Glycosmis pentaphyl la,* showed significant mortality at higher concentrations while the extract of *Acanthus ilicifolius,* executed insignificant mortality at the doses tested. The toxicity was possibly caused by the bioactive compounds present in the leaves of the selected plants.Many of these compounds are insect repellents or act to alter insect feeding behavior, growth and development, ecdysis and behaviour during mating and oviposition. A previous study showed the effect of pulverized leaves of *Annona squamosa* against the stored grain pest of wheat, *Tribolium castaneum.* In the study

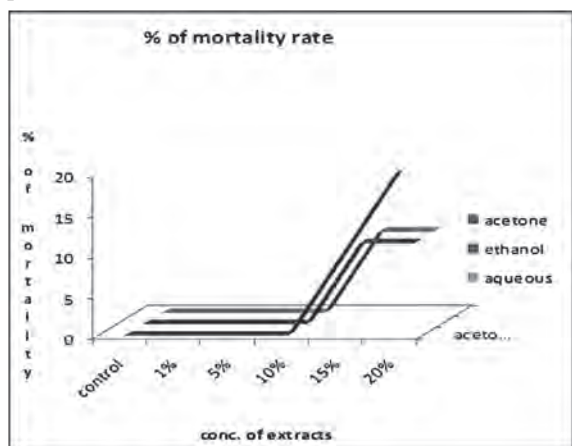

Fig.2. Effect of different extract of *Acanthus ilicifolius* on adult insects of *Sitophilus oryzae*

mortality rate increased with increase in concentration and resulted in 100% mortality with the highest concentration used [2].

Use of botanicals is currently promising and vital means to be used in the protection of crop products and prevent from polluting environment, which is a global problem. The mortality observed in the present study shows that insecticidal properties of leaves from the locally available plants with themselves having biologically active compounds offer means to safeguard the stored food grains from pests. This study arise scope for further investigations on the use of botanical products as alternatives of synthetic chemical pesticides in the effective control of the serious pest *Sitophilus oryzae*. Evaluation of the cost/benefit ratio regarding the use of ethano chemicals in the integrated pest management schemes and its effects on non-target organisms like mammalian toxicity studies can also be focused in future research.

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SNP Analysis and Predict SNP Analysis of MSH2 Gene

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Abstract

To understand the role of MSH2 gene product in relation to HNPCC disease we have analyzed the single nucleotide polymorphism (SNPs) associated with this gene. This helps us understand the genetic variation that can alter the function of the gene products. A total of 7 SNPs are investigated for MSH2 gene and colorectal cancer. To determine whether a missense mutation SNP in this gene affects its protein products. We use certain computational tools which predict 3 deleterious phenotypic effects. This polymorphic variation or mutations result in amino acid substitution from R to P at 524 positions and P to L at 622 positions.

Key words: MSH2, Single Nucleotide Polymorphism (SNP), Colorectal Cancer (HNPCC), Hereditary Nonpolyposis

Introduction

Most cases of colorectal cancer appear to be caused by somatic mutations, but a small number of cases are the result of germline mutations. The two most common inherited colorectal cancer syndromes are Hereditary Nonpolyposis Colorectal Cancer (HNPCC), caused by germline mutations in DNA mismatch repair (MMR) genes, and Familial Adenomatous Polyposis (FAP), caused by germline mutations in the tumour-suppressor gene adenomatous polyposis coli (APC) [9]. Studies of colon cancer in HnPCC families first identified MSH2 mutations in the MMR genes affected members of this cancer syndrome exhibited germ-line mutations, and individuals with sporadic colon cancer also exhibited somatic mutations in the MMR genes. Between 1990 and 2010[17].The existence of MSH2 germline mutations that substants altered the predicted gene product and co segregated with disease in the HNPCC kindred's that originally established linkage of HNPCC to chromosome 2[10]. The MSH2 gene provides instructions for making a protein that plays an essential role in DNA repair. This protein helps fix mistakes that are made when DNA is copied (DNA replication) in preparation for cell division. The MSH2 protein joins with one of two other proteins, MSH6 or MSH3 (each produced from a different gene), to form a protein complex. This complex identifies locations on the DNA where mistakes have been made during DNA replication. Another group of proteins, the MLH1-PMS2 protein complex, then repairs the errors. The MSH2 gene is a member of a set of genes known as the mismatch repair (MMR) genes14. MSH2 is homologous to the E. coli MutS gene and is involved in DNA mismatch repair (MMR) .Heterozygous mutations in the MSH2 gene result in hereditary nonpolyposis colorectal cancer-1 (HNPCC1; 120435). Epigenetic silencing of MSH2 caused by deletion of 3-prime regions of the upstream EPCAM gene (185535) and intergenic regions results in hereditary nonpolyposis colorectal cancer (HNPCC8; 613244). Alteration of MSH2 is also involved in Muir-Torre syndrome (MRTES; 158320) and mismatch repair cancer syndrome (MMRCS; 276300)[7].

Studies also show that about half of the SNP mutations occurring in the coding regions are missense while the rest are silent [8]. Since missense mutations are known to be one the main causes for major genetic disorders, many of these are the single causative factors for rare single gene inherited disorders. It is also expected that some more

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frequent missense mutations arising from SNPs in the coding regions will be associated with common genetic disorders [4].

DbSNP contains 1,463,178 submissions from 97 registered groups describing variation in five species human, mouse rat, chimpanzee, and the malaria parasite. Submission can be divided into four general categories with the following percentages of the total database size: (i) SNP mining from the human genome project sequences, 65%; (ii) private investigator/corporate experimental result, 28%; (iii) mined from EST databases, e.g. (Masood, 1999)13, 6%; (iv) continuing result of the NHGRI SNP discovery RFA, 1%. Public and private initiatives to discover new SNP in human identified over 306,000 variations in the period 1999-2000[5].

Materials and Methods

National Centre for Biotechnology dbSNP (SNP database)

The Single Nucleotide Polymorphism Database (dbSNP) is a free public archive for genetic variation within and across different species developed and hosted by the National Center for Biotechnology Information (NCBI) in collaboration with the National Human Genome Research Institute (NHGRI). Although the name of the database implies a collection of one class of polymorphisms only (i.e., single nucleotide polymorphisms(SNPs), it in fact contains a range of molecular variation: (1) SNPs, (2) short deletion and insertion polymorphisms (indels/DIPs), (3) microsatellite markers or short tandem repeats (STRs), (4) multi nucleotide polymorphisms (MNPs), (5) heterozygous sequences, and (6) named variants. The dbSNP accepts apparently neutral polymorphisms, polymorphisms corresponding to known phenotypes, and regions of no variation. It was created in September 1998 to supplement GenBank, NCBI's collection of publicly available nucleic acid and protein sequence dbSNP had amassed over 184 million submissions representing more than 64 million distinct variants for 55organisms, including Homo sapiens, Mus musculus, Oryza sativa, and many other species16. dbsnp database from NCBI was used for data curation. Gene name and disease name was typed in search engine to identify all SNPs related to gene.

PREDICTSNP

Consensus classifier that combines six of the top performing tools for the prediction of the effects of mutation on protein function. The obtained results are provided together with annotations extracted from the Protein Mutant Database and the UniProt database. PredictSNP consensus prediction was calculated using the following equation:

Table 1. SNP results from dbSNP

SNP ID	Chr position	SNP to chr	Function	SNP to mRNA	Position (mRNA)	Allele change	Protein position	Accession	Residue change
rs4987188	47416318	Fwd	Missense	Fwd	1037	GGC⇒GAC	322	XP_005264389.1	G[Gly] ⇒ D[Asp]
rs28929483	47475130	Fwd	Missense	Fwd	1937	CCA⇒ CTA	622	XP_005264389.1	P[Pro] ⇒ L[Leu]
rs63751108	47429881	Fwd	Stop Gain	Fwd	1288	CGA⇒TGA	406	XP_005264389.1	R[Arg] ⇒ Ter[*] [OPA]
rs63751207	47466718	Fwd	Missense	Fwd	1643	CGT⇒ CCT	524	XP_005264389.1	R[Arg] ⇒ P[Pro]
rs121908439	75032069	Rev	nearGene-3	NA	NA	NA ⇒ NA	NA	NA	NA

Table 2. Table indicating 2 highly deleterious mutations screened by predictsnp.

rs ID	Mutation	Nucleotide change	Polyphen 1	Polyphen 2	MAPP	PhD SNP	SNAP	Predict SNP
rs63751207	R542P	G/C	59%	55%	72%	88%	72%	87%
rs28929483	P622L	C/T	74%	68%	77%	88%	72%	87%

$$PredictSNP\ score = \frac{\sum_{i=1}^{N} (\delta_i \cdot S_i)}{\sum_{i=1}^{N} S_i}$$

Where N is the number of integrated tools represents the overall prediction (+1 for the deleterious prediction, −1 for the neutral prediction) and expresses the transformed confidence scores. The output value of PredictSNP score belongs to the continuous interval <−1, +1>. The mutations are considered to be neutral for the values in the interval <−1, 0> and deleterious for the values in the interval 0, +1>. The absolute distance of the PredictSNP score from zero expresses the confidence of the consensus classifier about its prediction. For easy comparison with the confidence scores of individual integrated tools, we transformed the confidence of the PredictSNP consensus classifier to the observed accuracy in the same way as described for confidence scores of the integrated tools[2].

Results and Discussion

A total of 7 SNPs were retrieved from dbSNP. Limits were activated to obtain SNPs 'Homosapiens' 'snp'.5 SNPs are found in MSH2 gene of humans. The table shows the SNP result from dbSNP (Table1).

The SNP associated with MSH2 were obtaining from the Single Nucleotide Polymorphism database [16]. Total of 10948 SNPs ids were retrieved for MSH2 gene, Limits were used for colorectal cancer we got only 7 SNPs for that again limits were used for getting the Homosapiens, finally 5 SNPs is got and thus MSH2 gene SNPs is filtered and it can be used for further analysis[12].

There are total of 5 SNPs were retrieved from the MSH2 gene, (rs4987188, rs28929483, rs121908439, rs63751207, rs63751108) here it contain different chromosome position and 4 Fwd and 1 Rev SNP to Chromosome direction is found with 3 missense function, 1 stop gain and 1 near gene, 4 Fwd direction with different position in mRNA allele change was occurred in 4 SNPs id [14]. The 4 snps contain 4 residue changes; there the MSH2 gene residue change was shown in different amino acid.

PREDICTSNP

Human active SNPs obtained from dbSNP database were uploaded in PredictSNP to find the deleterious SNPs. 3 SNPs were found to be deleterious. The table indicating the 2 highly deleterious mutations (Table 2)

Detection of deleterious nsSNPs is a major and challenging task in cancer studies. Here we identified 2 highly deleterious mutations screened by predictsnp are R524P and P622L as nucleotide change as G/C and C/T with rs ids of (rs63751207, rs28929483) respectively and that shows the highest percentage of 87% predictsnp. Most of the deleterious nsSNPs are well supported for the significant finding of the analysis[1, 3, 6].

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Biochemical Characterization and 16S rDNA sequencing of Lipolytic bacterial isolates WCS_1C_2 and WCS_3C_2 from windrow compost

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Abstract

16S rDNA sequencing has played a pivotal role in the accurate identification of bacterial isolates and the discovery of novel lipase producing bacteria from windrow compost for industrial applications. Two best lipase producing strains isolated from windrow compost were characterized by morphological, physicochemical characteristics and 16s rDNA sequencing. The genomic DNA extracted from WCS_1C_2 and WCS_3C_2 from the windrow compost were purified separately and used them as templates for the amplification of 16S rDNA gene sequences using Eppendorf thermal cycler. These 16S rDNA amplicaons were purified and subjected to automated DNA sequencing on ABI 3730xl genetic analyzer. The subsequent forward and reverse sequences of the 16S rDNA genes were aligned to obtain the consensus sequences that were analyzed with BLASTn using NCBI GenBank database. The multiple alignment analysis of the ten best chosen bacterial strain sequences with the respective WCS_1C_2 and WCS_3C_2 sequence was performed using Clustal X and the results were processed to make the phylogenetic tree using Treeview and NJ Plot. 16S rDNA sequence data revealed that the strain WCS_1C_2 had highest homology (99%) with *Staphylococcus saprophytics* and strain WCS_3C_2 showed highest homology (91%) with *Pseudomonas otitidi*s. The result of the present study indicated scope for utilizing lipase producing bacteria for further enhanced production of lipase, purification, characterization and various industrial applications.

Keywords: 16S rDNA, *Staphylococcus saprophytics,Pseudomonas otitidis*, lipase, Tributyrin, windrow compost.

Introduction

Lipases (EC.3.1.1.3, triacylglycerol acylhydrolases) are a group of enzymes, which have the ability to hydrolyze triacylglycerols at an oil-water interface to release free fatty acids and glycerol[1]. It also catalyzes a variety of organic transformations which are chemo-, regio-, enantio selective besides esterification under reduced non-aquous Conditions[2, 3]. Microbial lipases are high in demand due to their specificity of reaction, stereo specificity and less energy consumption than conventional methods[4]. Lipase has a large application in biotechnology. Bacterial lipase has been widely used in the food processing industry to hydrolyze the milk, fat milk, cheese ripening, improved synthetic flavors, and the process of lipolysis in the manufacture of cheese and cream[5]. Lipase had been also used in the detergent industry as an additive in powdered cleanser[6], in the textile industry to improve the absorbance of the product[7], in the synthesis of compounds or biodegradable polymers[8] and differentiation trans-esterification reaction[9]. This enzyme was used as a catalyst for the production of cosmetics[6], the paper industry[10], the synthesis of biodiesel[11], synthetic leather manufacturing industry, and the pharmaceutical industry[12]. Due to such multifaceted properties, it is one of the important industrial enzymes that have found usage in a wide array of industrial applications, such as food, detergent, and chemical industry and also in biomedical sciences. There is growing interest in large scale purification of lipases. Lipases from a large number of bacterial,

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fungal and a few plant and animal sources have been purified to homogeneity. Lipase producing bacterial sources is enormous but only few wild or recombinant strains have been used for commercial production of lipases.

Lipase-producing microbes could be found in habitats that support them such as industrial waste, oil processing plants, and oil-contaminated soil[13], such as solid waste dumping sited. At present, the management of organic waste is a major concern worldwide, as unscientific disposal of waste can adversely affect the environment by causing offensive odor, ground water contamination and soil pollution[14] .Thus, in recent years, vermicomposting and windrow composting have turned out to be promising way out for safe disposal of organic waste. Composting is an aerobic microbiological process that is facilitated by bacteria and fungi. Composting is also a method to produce fertilizer or soil conditioner. Both bacteria and fungi are present and active in a typical composting process[15] . Bacterial species composition was determined by isolating total DNA followed by amplifying and sequencing the gene encoding the 16S ribosomal RNA[16]. Among the different solidwaste management techniques Windrow compost is a good source of lipolytic enzyme producing bacterial load in both mesophylic and thermophylic stages[17].The use of 16S rDNA gene sequences to study bacterial phylogeny and taxonomy has been by far the most common housekeeping genetic marker used for a number of reasons. These reasons include (i) its presence in almost all bacteria, often existing as a multigene family, or operons; (ii) the function of the 16S rDNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and (iii) the 16S rDNA gene (1,500 bp) is large enough for informatics purposes[18].

The present study deals with the bacterial identification using 16S rDNA sequencing to find out novel lypolytic isolates from windrow composting and to traces its phylogeny. Further purification and isolation of novel strains can be used to augment biotechnology industry.

Materials and Methods

Collection of the compost sample

Windrow compost was done in solid waste management unit of Mercy college campus. The compost bed was prepared by kitchen food waste generated at Mercy college campus. Kitchen food waste and cow dung inoculums in the ratio 10: 1is spread in alternative layers, forming a windrow. Compost samples were collected at an interval of 10 days for about six turns. About 10g of soil samples were collected using a sterile spatula in a sterile beaker. Temperature and pH of the sample were recorded.

Isolation of lipase producers

The compost samples were collected from different turns of the compost bed located at Mercy College, Solid waste management unit, Kerala enriched by periodic sub culturing of samples in Nutrient Broth (NB) media. They were aseptically subjected to serial dilutions and plated on Nutrient Agar (NA) and incubated at 37°C for 24, 48 and 72 h. Acceptable plate counts for bacteria were between 30 – 300 cfu/ml per plate. After incubation 73 predominant bacterial colonies were isolated and screened for lipase activity and then subjected to morphological, cultural and biochemical examinations.

Screening for lipase activity by Tributyrin Clearing Zone (TCZ)

The predominant bacteria in the nutrient agar plate were isolated and screened for lipolytic activity. Lipolysis is observed directly by changes in the appearance of the substrate such as tributyrin, which are emulsified mechanically in various growth media and poured into a petri dish. The bacterial isolates were screened for lipolytic activity on agar plates containing tributyrin (1%, w/v), agar (2%, w/v) in Luria–Bertani medium. Lipase production is indicated by the formation of clear halos around the colonies grown on tributyrin- containing agar plates[19, 20].

Characterization of Bacterial lipase producer - Tributyrin Plate Assay

The bacterial isolates were screened for lipolytic activity on tributyrin agar plates. A loopful of isolate was streaked into the tributyrin medium and incubated at 37°C for 24 hours. After incubation the isolates were observed for lipolysis i.e zone of hydrolysis around the colony. Lipase production is indicated by the formation of clear halos around the colonies grown on tributyrin- containing agar plates. Halos around the colonies on tributyrin agar plates are considered as positive colonies for lipase

enzyme production. Such colonies are isolated and identified by phenotypic characterization based on morphological, biochemical and physiological characters according to Bergeys Manual of Systematic Bacteriology[21]. Characterization and identification of the isolate with higher lipolytic activity was carried out both biochemically and by 16s r RNA sequencing.

Morphological Characterization

All of the isolates that showed positive lipolytic activity on the screening medium were further characterized on the basis of microscopic appearance and cultural characteristics. The morphology of the isolates was observed microscopically to deduce their responds towards Gram staining, spore staining and acid fast staining test. The evaluations based on the shape, size, elevation and opacity were also done towards the colonies characteristics after 48 hours incubation on selective medium. In addition, motility agar stab was prepared to determine the existence of flagella in the selected bacterial isolates[22].

Conventional biochemical test methods

The following biochemical tests were done on all the bacterial isolates that showed positive lipolytic activity: oxidase, starch hydrolysis, V-P reaction, catalase, urease, citrate, carbohydrate utilization, indole production and methyl-red test. All biochemical tests were conducted according to standard and established biochemical charts[23]. Lipolytic bacterial isolates were identified up to the species level by ABIS online software.

DNA isolation and 16rDNA Amplification

Genomic DNA was extracted using a genomic purification kit (Promega, USA).16S rDNA fragment was amplified by PCR from the above isolated genomic DNA. A single discrete band was observed when resolved on Agarose Gel. The PCR amplicon was purified by column purification in order to remove contaminants. DNA seqeuncing of PCR amplicon. DNA was then amplified by PCR using the following universal 16S rDNA gene primers, (8F: 5'-AGAGTTTGATCMTGG-3') as forward primer and (1492R: 5'-ACCTTGTTACGACTT-3') as reverse primer. PCR was carried out in a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube. The PCR tubes with all the components were transferred to thermal cycler. The PCR was conducted by subjecting a reaction mixture to initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, Anealing 58°C for 30 seconds , Extension 72°C for 45seconds and a final elongation step at 72°C for 10 min. The presence of PCR products was determined by electrophoresis of 10 µLof the reaction product in a 1% agarose gel. Commercially available 100bp ladder was used as standard molecular weight DNA. Amplified PCR product was purified using column purification as per manufacturer's guidelines, and further used for sequencing reaction.

Sequencing of Purified 16S rDNA Gene

The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on ABI3730xl Genetic Analyzer (Applied Biosystems, USA). Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequence at the 3'and 5' ends (considering peak and Quality Values for each base) using the sequence analysis tools. The edited sequences (16S rDNA) were then used for similarity searches using BLASTn (Basic Local Alignment Search Tool) programme in the NCBI GenBank (www.ncbi.nlm.nih.gov) DNA database for identifying the bacterial strains.

Phylogenetic analysis

The 16S rDNA gene sequence was compared with sequences available in the nucleotide database using the Clustal X(1.8.1) and Phylogenetic tree was constructed using the neighbor joining method (http://www.phylogeny.fr) The cladogram was plotted using Treeview and NJ Plot sotware.

Results and Discussion

Lipases are currently used in different industrial products and processes and new areas of applications are constantly being added, which include the production of single cell protein, cosmetics, pulping, lubricants etc[24]. Lipase producing microbes have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds, and decaying food , compost heaps, coal tips, and hot springs[25]. The bacterial cultures were isolated from different turns of the windrow composting process from Mercy College campus solid waste units[17]. Molecular tools like 16S rDNA and COMPOCHIP

microarray were used to study microbial community shifts during the coffee husk composting process[26]. Enzyme Production-Based Approach for Determining the Functions of Microorganisms within a Community during the composting process have been attempted earlier[27].

Screening and Isolation of Lipolytic bacteria

A total of 73 bacterial cultures was obtained from the enriched samples by dilution technique using nutrient agar medium (Table.1).

Table 1. Bacterial Cultures obtained from Different turns of windrow compost

Windrow compost turns	Isolate code	Total Number of bacterial cultures
1	WCS_1C_1	10
2	WCS_2C_1	14
3	WCS_3C_1	16
4	WCS_4C_1	16
5	WCS_5C_1	12
6	WCS_6C_1	5

Note: WC-windrow compost

These bacterial cultures were repeatedly sub-cultured to obtain pure cultures and were maintained on nutrient agar slants at 4°C. They were screened for lipase activity by cultivating them on tributyrin agar medium and observed for the presence of clear halos (zone) around the colonies. Among the 73 isolates 24 showed clear zone in the tributyrin medium. The bacterial isolate which showed lipolytic activity was screened for lipase production in the screening medium. The bacterial isolates WCS_1C_2 and WCS_3C_1 which showed maximum lipase production was further characterized and identified by morphological, biochemical characteristics and by 16s rDNA sequencing .

Phenotypic Characterization

a) Morphological characteristics

The results on colony morphology and microscopic observation of the selected bacterial culture WCS_1C_2 and WCS_3C_1 are presented in Table 2. The results showed that the colony morphology of the bacterial culture WCS_1C_2 appeared to be large, entire, very glossy, smooth, opaque, butyrous, convex, usually white but colonies can be yellow or orange on nutrient agar medium. The microscopic observation of differentially stainedbacterial culture WCS_1C_2 showed that the bacterium is cocci, gram positive, non-spore forming and arranged in Grapelike clusters.Colony morphology of the bacterial culture WCS_3C_2 appeared to be small,-circular,raised with undulate margins ,gramnegative rods with single flagellum.

Biochemical characteristics

The results on biochemical characteristics of the selected bacterial cultures WCS_1C_2 and WCS_3C_2 were presented in Table.3. The bacterial culture WCS_1C_2 is positive for catalase, MR, VP,Hydrogen production, urease and nitrogen reduction

Table.2 Morphological Characteristics of Bacterial Cultures WCS_1C_2 and WCS_3C_1

Characteristics	*Staphylococcus saprophyticus*	*Pseudomonas otitidis*
Colony morphology		
Size	2mm	1.5 – 5.0μm.
Color	White yellow	cream
Form	spherical	circular
Surface	entire	concave
Texture	Opaque, glossy	glossy
Elevation	convex	raised
Margin	Wavy Margin	undulate
Microscopic observation		
Gram stain	Gram-positive	negative
Shape	cocci	rod
Size	2mm	1.5 – 5.0μm.
Arrangement	Grapelike clusters	single
Spore formation	non-spore forming	non-spore forming

Table 3. Biochemical Characteristics of the Bacterial Cultures WCS_1C_2 and WCS_3C_2

No	Biochemical test	WCS_1C_2 (*Staphylococcus saprophyticus)*	WCS_3C_2 (*Pseudomonas otitidis*)
1	Catalase test	+	+
2	Oxidase test	-	+
3	Indole Production test	-	-
4	Methyl Red test	+	+
5	Voges Proskauer test	+	-
6	Citrate utilization test	-	+
7	Macconkey agar test	-	+
8	Manitol test	+	+
9	Motility test	-	+
10	Hydrogen production test	+	-
11	Urease test	+	-
12	KOH test	-	-
13	Acetate utilization test	-	-
14	Lactose fermentation test	-	+
15	Starch hydrolysis test	-	-
16	Gelatin hydrolysis test	-	+
17	Nitrate Reduction test	+	+
18	coagulase	-	-
19	Haemolysis	alpha	-

test.nitrate and citrate tests. Biochemical tests like catalase,oxidase,MR, Citrate utilization etc were found to be positive in the case of bacterial culture WCS_3C_1. Based on morphological and biochemical characteristics the bacterial culture WCS_1C_2 showed typical characteristics of genus *Staphylococcus* and WCS_3C_2 belongs to genus *Pseudomonas*.

Molecular Characterization

16S rDNA gene sequencing Analysis

The 16S rDNA gene was amplified and the resultant gene product of WCS_1C_2 isolate was found to be 805 bp and of WCS_3C_2 465bp.The PCR product of 16S rDNA gene was separated in gel as single bands (Fig 1.).For the past few decades, sequencing of 16S rDNA genes are serving as tools for bacterial detection and identification[28]. The nearest neighborhood bacterial strains were analysed by BLASTn and were tabulated in Table4, 5 and 6. 805 bp of the 16S rDNA genes of strain WCS_1C_2 showed 99% similarity with *Staphylococcus saprophyticus* with Accession No: KT717631.1 in the Genbank and 465 bp of the 16S rDNA genes of strain WCS_3C_2 showed 91% similarity with *Pseudomonas otitidis* AAIII-6. (Accession No: LN558619.1) in the genbank

Phylogenetic tree construction

To identify the bacterial isolate, representative sequences with 99% identity from BLASTn (Table 4 and 5) were retrieved from NCBI and aligned using CLUSTAL X(1.8.3). Among the nine representatives used in the analysis, for bacterial culture WCS_1C_2 showed 99% similarity towards *Staphylococcus saprophyticus* strain PbT7 (KT717631.1)(Table.6) which was evident in the rectangular cladogram (Figure2a)and NJ plot(Fig.2b). Hence, the culture under study with following code WCS_1C_2 was conclusively confirmed as *Staphylococcus saprophyticus*. The nearest neighborhood strain of

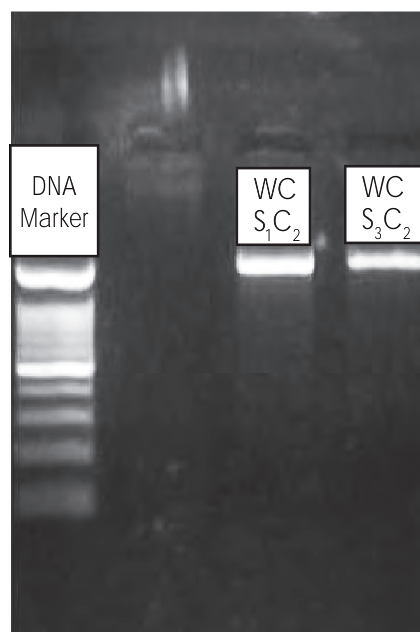

Fig. 1. Visualization of amplified16S rDNA fragments of 100bp of microbial isolates WCS_1C_2 and WCS_3C_2

Table.4. Isolate WCS_1C_2 *(Staphylococcus saprophyticus)* homology to the nearest known neighbor hood bacterial strains

No	Accession Number	Bacterial Strain	Max score	Total score	Query cover-age	E val-ue	Max iden-tity
1	KC853118.1	*Staphylococcus* sp. Ac15	1482	1482	100%	0.0	99%
2	KU725839.1	*Staphylococcus saprophyticus* strain 18_2e	1482	1482	100%	0.0	99%
3	KT717631.1	*Staphylococcus saprophyticus* strain PbT7	1482	1482	100%	0.0	99%
4	KT441087.1	*Staphylococcus saprophyticus* strain IARI-CRK 5	1482	1482	100%	0.0	99%
5	KT441038.1	*Staphylococcus saprophyticus* strain IARI-BGL 14	1482	1482	100%	0.0	99%
6	KT372841.1	*Staphylococcus saprophyticus* strain T54	1482	1482	100%	0.0	99%
7	KT372851.1	*Staphylococcus xylosus* strain T47 16S ribosomal RNA gene	1482	1482	100%	0.0	99%
8	KT372842.1	*Staphylococcus saprophyticus* strain T63 16S ribosomal RNA gene, partial sequence	1482	1482	100%	0.0	99%
9	KT159438.1	*Staphylococcus saprophyticus* strain Na42RB-2 16S ribosomal RNA gene, partial sequence	1482	1482	100%	0.0	99%
10	KP296225.1 Na49RA	*Staphylococcus saprophyticus* strain 1 16S ribosomal RNA gene, partial sequence	1482	1482	100%	0.0	99%

WCS_3C_2 may be considered as the *Pseudomonas otitidis* AAIII-6, isolated from rivers, lake and

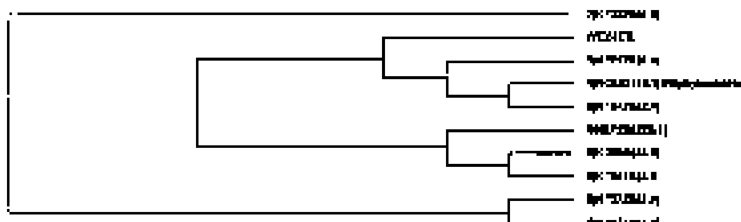

Fig.2 a. Rectangular Cladogram based Phylogenetic position of 16S rDNA gene sequence of the strain WCS_1C_2 among different strains of *Staphylococcus saprophyticus*

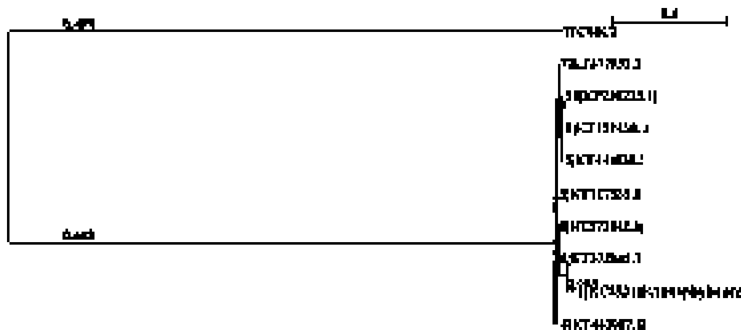

Fig.2 b. NJ plot based Phylogenetic position of strain WCS_1C_2 among different strains of *Staphylococcus saprophyticus.* The evolutionary lineage of 0.1 shows 10 mutations/100 nucleotides amomg *staphylococcus* sps.

Table. 5 Isolate WCS_3C_2 (*Pseudomonas otitidis*) homology to the nearest known neighborhood bacterial strains

No	Accession Number	Bacterial Strain	Max score	Total score	Query coverage	E value	Max identity
1	LN558619.1	*Pseudomonas otitidis* partial 16S rRNA gene, isolate AAIII-6	610	610	97%	8e-171	91%
2	KT759070.1	Bacterium DD10 16S ribosomal RNA gene, partial sequence	612	612	97%	2e-171	91%
3	LN558605.1	*Pseudomonas otitidis* partial 16S rRNA gene, isolate AAII-1	610	610	97%	8e-171	91%
4	LN558604.1	*Pseudomonas otitidis* partial 16S rRNA gene, isolate AAI-10	610	610	97%	8e-171	91%
5	LN558600.1	*Pseudomonas otitidis* partial 16S rRNA gene, isolate AAI-6	610	610	97%	8e-171	91%
6	KF159806.1	*Pseudomonas otitidis* strain JY6 16S ribosomal RNA gene, partial sequence	610	610	97%	8e-171	91%
7	GU204968.1	*Pseudomonas otitidis* strain RW1	610	610	97%	8e-171	91%
8	NR_043289.1	*Pseudomonas otitidis* strain MCC10330	610	610	97%	8e-171	91%

Table.6 Data analysis on NCBI website using BLASTn

Bacterial isolates	No. of nucleotides	Identity (%)	Sequence Closest phylogenetic relative (GenBank accession no.)
WCS_1C_2	805	99%	*Staphylococcus saprophyticus* strain PbT7(KT717631.1)
WCS_3C_2	465	91%	*Pseudomonas otitidis* AAIII-6(LN558619.1)

Fig.3a Phylogenetic position based on 16S rDNA gene sequence analysis of strain WCS_3C_2 among different strains of Pseudomonas otitidis

spring water in Belgrade, Serbia which is also evident from the phylogenetic tree shown in the Figure3a&b.

Lipase produced from Bacillus pumilus[29,30] from oil-containing wastewater were reported. Phylogenetic analysis based on 16S rRNA genes and biochemical characterization allowed identification

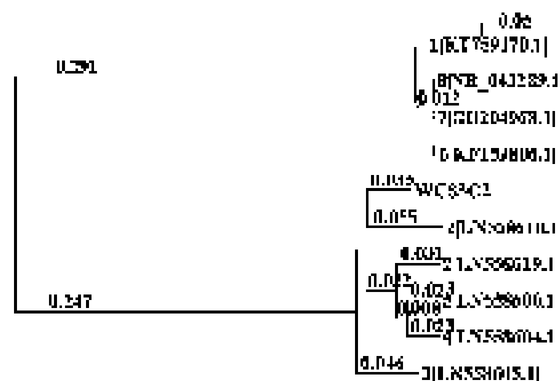

Fig3b. NJ plot based Phylogenetic position of strain WCS_3C_2 among different strains of pseudomonas otitidis. Defines 5 nucleotide substitution had occurred per 100in the pseudomonas otitidis.

of four different species belonging to four genera: *Geobacillus thermodenitrificans, Bacillus smithii, Ureibacillus suwonensis* and *Aneurinibacillus thermoaerophilus* from manure compost[31]. A new strain

of *Pseudomonas aeruginosa* KM110 was similarly isolated from wastewater of an oil processing plant by16S rRNA sequencing[32] .The acquired 16S rDNA gene sequences were analyzed again using analysis software RDP Classifier in order to assign them into their respective phylogeny and bacterial taxonomy[28].

Based on the displayed results, it was concluded that WCS_1C_2 isolate belongs to the members of Bacteria domain, Firmicutes phylum, *Bacilli* class, *Bacillales* order and Family *Staphylococcaceae* and genus *Staphylococcus*. While the other isolate WCS_3C_2 assigned to be the members the members of kingdom Bacteria, Proteobacteria phylum, Gamma Proteobacteria class, *Pseudomonadales* order and Family Pseudomonadaceae and genus *Pseudomonas*. These results were correlated with the BLASTn results where it aligns the 16S rDNA sequences of the isolates WCS_1C_2 specifically to *Staphylococcus saprophyticus* with 99% identity and WCS_3C_2 to *Pseudomonas otitidis* with 91% identity. The homologous organisms for isolate WCS_1C_2 as shown in the Table-4 and 6 were of maximum similarity (99%) to the genus *Staphylococcus* genus having the similar characters to its nearest neighbors *Staphylococcus saprophyticus* strain PbT7(KT717631.1) that were isolated from Pb tolerant *Staphylococcus* species isolated from metal contaminated soil at Zacatecas, Mexico. The nearest neighborhood strain of WCS_3C_2 may be considered as the *Pseudomonas otitidis* AAIII-6, isolated from rivers, lake and spring water in Belgrade, Serbia. The result of the present study indicated scope for utilizing lipase producing novel bacteria from windrow compost for industrial applications from the cheapest source.

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Screening of mutation in *BRCA* genes and prediction of cancer susceptibility and genetic risk assessment

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Abstract

Breast cancer is the most common cancer seen in women worldwide, early detection makes cancer curative. Present study gives a clear image of breast cancer susceptibility among family even if it is in a single gene mutation. By blood DNA analysis 'first hit' of mutation can be analyzed through conformational sensitive gel electrophoresis. This information generated by CSGE may create awareness so that the high risk individual can adopt a suitable lifestyle to prevent cancer incidence. It also helps physicians and genetic counselors in helping women facing positive result of breast cancer for taking available preventive options.

Keyword: Breast cancer, BRCA, CSGE.

Introduction

Cells are the basic unit of life. Normally the cells grow and multiply when and where they need and apoptosis in correct time. But a cancer cell divides uncontrollably and doesn't undergo apoptosis. This leads to the formation of tumor. Mutations in a number of genes are now known to cause susceptibility to breast and/or ovarian cancer. In the context of high-risk families, the most important genes are BRCA1 and BRCA2. BRCA1 was localized to chromosome 17q by genetic linkage in 1990[1] and subsequently was cloned in 1994[2]. Studies to date suggest that BRCA1 accounts for the majority of families containing multiple cases of breast and ovarian cancer[3&4]. BRCA2 was localized to chromosome 13q in 19945 and was cloned in 1995[6].

Recent years have marked important progress in understanding inherited susceptibility of breast cancer. With the identifications of the BRCA1 and BRCA2 genes, inherited mutation of one of these genes results in making its carriers at a much increased risk of developing breast cancer as well as ovarian cancer and it has become possible to test for the presence of these mutations. Although the availability of screening represents a major opportunity both scientifically and clinically, it also creates ample opportunities for patients for better prevention[7]. The chance of carrying a genetic mutation varies markedly from woman to woman, depending on family history of breast cancer and related cancers. As a result, increasing attention needs to be given to understanding and conveying risk information in an individualized way. Testing for BRCA1 and BRCA2 mutations is expensive, and a positive outcome can affect a person's life in important ways: ineligibility for health insurance and in potential employment discrimination, as well as in physical and psychological aspects. A positive test or simply the perception of a high risk can lead to aggressive management, ranging from more-frequent mammographies to bilateral mastectomy, again with substantive consequences on a woman's life.

The purpose of this work is to describe a method for finding the probability that a particular family member carries a germ-line mutation at BRCA1 or BRCA2, on the basis of her family's history of breast cancer. Although the method applies to both women and men, the victim is usually a female. The family history includes the patient and her first- and second-degree relatives. This study determines the mutation in BRCA gene of person by Conformational Sensitive Gel Electrophoresis (CSGE). Through CSGE it can be possible to analyses even

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a single germ line mutation in BRCA gene. CSGE is a technique which detects single base mismatches in DNA heteroduplexes[8]. There are at least two principles on which the CSGE method works. First, single-base mismatches can produce conformational changes such as a bend in the double- helix of the DNA, leading to the differential migration of heteroduplex and homoduplex. Second, mildly denaturing solvents in an appropriate buffer can accentuate the conformational changes produced by single-base mismatches, resulting in the increased differential migration of heteroduplexes and homoduplexes. The CSGE method involves heteroduplex analysis of PCR products in a novel, mildly denaturing polyacrylamide gel matrix using a different cross-linker, bis-acrolyl-piperazine, instead of the conventional bis-acrylamide.9 Essentially, the protocol involves amplification of the entire coding region in small fragments and analyzing them by CSGE. The presence of additional slow or differentially migrating bands in comparison to normal sample indicates the presence of heteroduplex bands, which are suggestive of presence of mutation. The samples that show heteroduplex bands have to be sequenced to locate and identify the nature of mutations.

Materials and methods

Collection of blood samples

2 to 3 mL of blood sample were collected in tubes containing EDTA and stored at -800C

Isolation of DNA from blood

Human DNA is usually isolated by digestion of cells with proteinase K in the presence of EDTA and a detergent such as SDS, followed by extraction with phenol.[9]

This method for DNA extraction involves cell lysis by mechanical disruption using Phosphate Buffered Saline (PBS) followed by degradation of protein with proteinase K. The EDTA present in the buffer inhibits the action of DNAase. The degraded proteins are extracted with phenol and chloroform. The DNA is then precipitated with NaCl and ethanol. The salt NaCl provides Na^+ ions which form an ionic bond with the negatively charged phosphates on DNA, neutralizing negative charges, thereby allowing phosphate ends to come closer so they can precipitate out in cold alcohol solution. Later the precipitated DNA is re suspended in TE (Tris EDTA) buffer.

DNA isolation

The blood sample was thawed in a water bath at room temperature and 0.5 mL of blood was aliquoted into a 1.5 mL microcentrifuge tube. Then an equal volume of PBS was added and centrifuged at 12000 rpm for 30 seconds the supernatant containing lysed red cells was discarded. Re-suspended the pellet in 0.5 mL of extraction buffer. Then the solution was incubated for 1 hour at 370C. Proteinase K to a final concentration of 100μg/mL was added to it and mixed gently by tapping with fingers.10The suspension of lysed cells was placed in a water bath for 3 hours at 500C. Swirled the viscous solution periodically.Then cooled the solution to room temperature. Added an equal volume of phenol equilibrated with 0.5 M TrisCl (pH 8). The two phases were gently mixed by slowly turning the tube end over for 10 minutes. The two phases were then separated by centrifugation at 12000 rpm for 1 minute. Using a wide bore pipette (0.3 cm diameter orifice) transferred the viscous aqueous phase into another 1.5 mL micro-centrifuge tube. An equal volume of chloroform was added and mixed gently for 10 minute. Then Centrifuged at 12000 rpm for 1 minute and transferred the aqueous phase into another 1.5 mL micro-centrifuge tube.

Precipitation of DNA

The volume of DNA solution was estimated and NaCl to a final concentration of 0.2m M was added and 2 volumes of ice cold ethanol was added and mixed well. Kept it in ice for 30 minute/overnight. Recovered the DNA by centrifugation at 40C at 12000 rpm for 15 min. The supernatant was carefully removed. Any drops that adhere to the walls of the tube were removed using a pipette tip. 0.5 mL 70% ethanol was added and centrifuged at 12000 rpm for 10 min at 400C and repeated step 4. After, stored the open tube on the bench at room temperature until the last traces of fluid had evaporated. Then dissolved the DNA pellet in 200 μL of TE.

Conformation sensitive gel electrophoresis (CSGE)

CSGE protocol-Pouring the gel

Glass plates and spacers were washed thoroughly with a house hold detergent, rinsed with double distilled water and dried with disposable tissues. Then cleaned the inner surface of the plates with 70% alcohol and wiped it dry.Applied Vaseline to

the spacers (1mm) and assembled the glass plates. The edges of the plates were also sealed with petroleum jelly. Placed the binder clips all around and the glass plates were strongly clamped over the spacers.130 mL of acrylamide solution was prepared at the required percentage and acrylamide- BAP cross linker ratio. Ammonium PerSulphate (APS) must be freshly prepared. 1.3mL freshly made 10% ammonium per sulphate and 74μL TEMED were added to the acrylamide solution in the beaker prior to pouring and swirled gently to mix. (Once the ammonium per sulphate solution and TEMED are added, polymerization commences and hence the gel must be poured immediately.) The assembled glass plates were placed at an angle of 450 on the bench and carefully filled the glass plates with acrylamide solution from a corner, avoiding air bubbles. Any air bubbles introduced while pouring could be brought to the meniscus by gently tapping on the glass plates. Then the comb was inserted, taking care not to trap any air bubbles and left the gel to polymerize at an incline for 90 minutes. During the first 15 minutes the gel was monitored for any leakage. (If leakage does occur, the gel can be topped up with excess acrylamide from the beaker.)After gel was polymerized, removed the binding clips and rinsed any acrylamide from the glass plates with tap water and removed the comb. The gel with glass plates were placed in the electrophoresis apparatus and firmly locked in place using the four locking screws. Ensured that the drain tap to the upper buffer chamber was closed and filled the upper and lower reservoirs with about 1000 mL of 0.5XTTE buffer.Flushed unpolymerized acrylamide from the wells with 0.5XTTE using a syringe and a fine gauge needle. The leads on the electrophoresis tank were connected to the power pack and a pre-run was given at a constant voltage of 750V for 60 minutes.

Hetero duplexing

The heteroduplex formation was enhanced by heating PCR products in the PCR machine as follows. Samples were heated at 980°C for 5 min and followed by incubation at 680°C for 1 hour. This step could be carried out during the last cycle of PCR if the samples are to be used for CSGE immediately. Alternatively, heteroduplexing reaction could be done just before loading the samples onto the gel.

Running the gel

After pre-running at 750V for 60 minutes, the wells were thoroughly cleaned again. (It is very important for the sharpness of the DNA bands that the wells are thoroughly cleaned before the pre-run and again after the pre-run prior to loading the samples). Loaded the gel with appropriate amount (typically 6–10 μL) of heteroduplexed PCR product mixed with 2–4μL of loading dye.Electrophoresed the gel for about 16 h at constant voltage (400 V) for 300 to 500 base fragments (for 38.5cm glass plate).

Reading the gel

Removed plates from gel apparatus and separated the small plate from the large plate.The gel was stained in 0.5 X TTE containing 0.05 mg/mL Ethidium Bromide solution for 5-7 minutes in large staining tray. Care was given to minimize background by not over staining. After staining, the gel was de-stained in distilled water for 10 minutes. Then the bands were visualized by ultra violet light in dark room with the aid of UV transilluminator. Then, the relevant portion of the gel was taken with a scalpel, transferred on Gel documentation system (Bio-Rad) and the images generated were saved in the computer. Samples displaying abnormal CSGE profiles compared to that of controls were identified. (Immediate photography is very important not only for the purpose of record but also to identify the heteroduplexes in cases where the heteroduplexes move very close to the homoduplexes.

Confirmation of mutations

Mutation of all genes are confirmed by sequencing data which show mutation in CSGE.

Resultand Discussion

Out of the 8 families selected for germline BRCA2 mutation analysis, 43 women were examined. The breast/ovarian cancer cases ranged from ages 22 to 79 years with a mean age of 42.88 years. Their DNA was isolated and CSGE was carried out. Out of these eight families, only one family show positive result in CSGE. Breast cancer patients, prevalence of gene mutation in exon 9 of BRCA 2 gene was detected in 3 generations of one family. Loss of heterozygous condition in the breast tissue due to mutations in this exon could be the reason for the mother and two daughter getting breast cancers, the other sibling and two

grandchildren remaining free of cancer as all the individuals carried the heterozygous condition in their cellular genes. One granddaughter is affected. In all this case sequencing reveals that missense mutations occurred in the same locus in exon 9. Which clearly indicate the inheritance of breast cancer among family.

Mutation analysis of BRCA2 gene in eight breast cancer family has identified germline alterations in four individuals in a family. Here a single base pair mutation is inherited throughout the family. This mutation frequency is in agreement with previous reports in other populations[12]. But studies on several other populations had reported comparatively higher pathogenic mutation frequencies for BRCA2 gene[13]. However, contradictory results among these studies might probably be because of differences

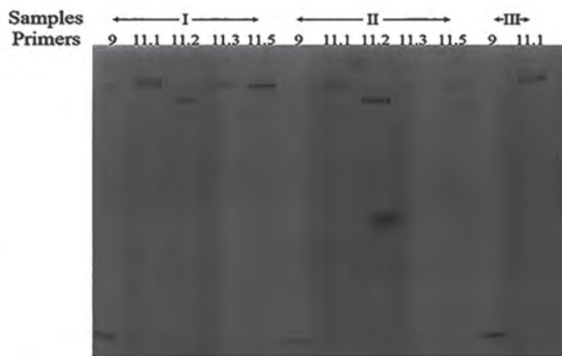

Fig.1 Gel showing no heteroduplex band in CSGE. CSGE of 3 (I, II and III) did not have any triple banding for all these primer (Absence of any mutation).

in the characteristics of the patient population analyzed, and criteria used for the selection of these families. Variation in the sensitivity of the screening techniques might also have accounted for the differences in the results. Additional variability in the results of population-based studies might also be attributed to the variable contribution of founder mutations in different ethnic populations.

The most commonly used strategy for detecting mutations in large and complex genes is to amplify sequences of genes of interest by PCR, scan the PCR products for the presence of mutations by a rapid procedure, and then sequence only those PCR products that were positive for mutation by the scanning technique to locate and to identify the nature of mutation. The most commonly used scanning techniques for PCR products are single-stranded conformation polymorphism (SSCP)[14], enzymatic or chemical cleavage of mismatched base pair[15] and differential unfolding of homo duplexes and heteroduplexes by denaturing gradient gel electrophoresis (DGGE). As per the currently available techniques for scanning PCR products, single-stranded conformation

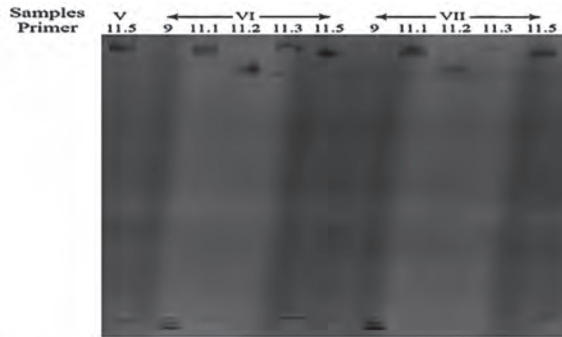

Fig.2 Gel showing heteroduplex band in CSGE. Sample IV, V, VI and VII showed double or triple banding for primer9 indicate a point mutation at 9th segment of BRCA-2.

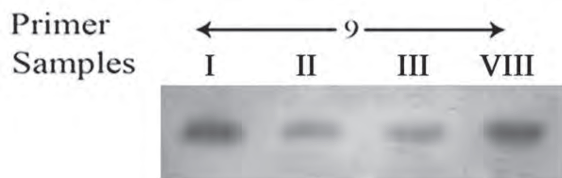

Fig.3 Normal banding

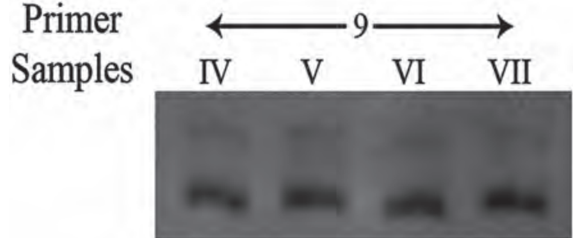

Fig.4. Mutated banding. Sequencing of the sample revealed a deletion of Adenine nucleotide at the 70763 position of the gene.

Note:

Sample IV EXON.9: CATTCTGCAAAA-TTATAGTTTAAAATCAC

Sample V EXON.9: CATTCTGCAAAA-TTATAGTTTAAAATCAC

Sample VI EXON.9: CATTCTGCAAAA-TTATAGTTTAAAATCAC

Sample VII EXON.9: CATTCTGCAAAA-TTATAGTTTAAAATCAC

BRCA2 reference: CATTCTGCAAAAATTATAGTTTAAAATCAC

TAGTATAAAAACTCTCAATG

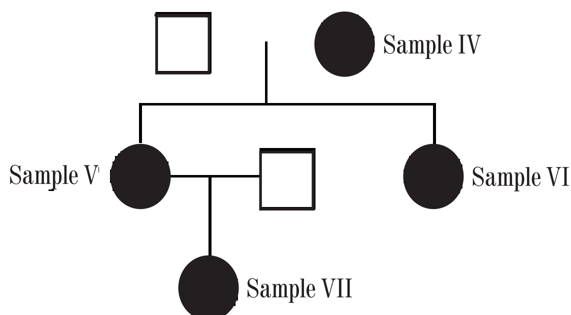

Fig.5 Pedigree showing positive family history of BRCA 2 gene.Pedigree analysis of the sample showed prevalence of gene mutation in exon 9 of BRCA-2 gene was detected in 3 generation of one family.

polymorphism analysis of polymerase chain reaction (PCR) products (PCR-SSCP) is commonly used for detecting mutations. However, this assay is not reliable with fragments of greater than about 200 bp, and the sensitivity is estimated to range from about 60% to 95%. Moreover, fragment labeling and product visualization, which was originally via radioisotope labeling and autoradiography in SSCP technique, warranted the need for newer techniques. On other hand, Conformation-Sensitive Gel Electrophoresis (CSGE), is very simple, very effective for larger-size fragments, and does not involve radioactivity. This method basically identifies heteroduplex bands, which arise due to annealing of complementary strands, one each from mutant and wild-type alleles. Another advantage is that in CSGE, Ethidium Bromide (EtBr) staining replaces radioisotopes and hence no radioactivity is needed as compared to SSCP, which allows greater freedom when performing the assay. It is also possible to put more than one sample (of varying sizes) in a single lane, which is usually difficult with the SSCP method due to the presence of multiple bands per sample. It offers a relatively simple method for mutation detection. This technique can be applied to any PCR products of 200-800 base pairs which limit the amount of labor required to screen the complete gene. It requires no special equipment or preparation of samples except for pouring a standard polyacrylamide gel in a modified solvent buffer system. Hence, CSGE is not costly and most long polyacrylamide gel electrophoresis systems can be adapted for this application. A single set of electrophoretic conditions have been determined which is thought to enable detection of 100% of sequence variants. Consumable costs are low. Results are easily interpreted. It is also a rapid technique.

Conclusion

Inheritance of one defective BRCA1 or BRCA2 allele sufficient to conform cancer predisposition. Breast and ovarian tumors from patients almost invariably exhibit loss of heterozygosity. Somatic mutations in BRCA1 or BRCA2 do not frequently occur in sporadic (non-familial) breast cancers but inherited breast cancer is linked to those of BRCA1 or BRCA2 gene mutation. So by analyzing heteroduplexing in BRCA gene through conformational sensitive gel electrophoresis predisposition of breast cancer can be confirmed.

These findings show the relative importance of specific family history and other characteristics in predicting mutation, and may serve to alert women to indicate potentially heightened likelihood of carrying a mutation. Of note, the results presented here summarize aggregate results for individual risk factors with respect to family history. Here out of 8 families, one family shows positive result of breast cancer inheritance. But, it should not be assumed that the presence (or absence) of any one factor in a woman's profile necessarily equates to a high (or low) likelihood of carrying a mutation. Lastly, this report is giving insights regarding the predictors of being a mutation carrier. These results also serve as a continued reminder that the majority of women with breast cancer, even those with a first-degree family history, do not carry mutations in these genes.

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A study of stomatal index based on the concept of evolutionary age of selected plants

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Abstract

Stomata are specialized structures found on the leaves for gas exchange in plants. A study was conducted to compare stomatal indices of selected primitive and advanced dicotyledonous and monocotyledonous plants of Thalassery during 2012. Plants belonging to Magnoliaceae, Annonaceae, Malvaceae, Rubiaceae, Asteraceae, Commelinaceae, Arecaceae, and Amaryllidaceae were selected and stomata of lower epidermis were observed under Magnus compound microscope. The study revealed a higher number of stoma is usually correlated to the recently evolved plants and a lower number of stoma is seen in primitive plants.

Key words: Stomatal index, evolution, respiration, gas exchange.

Introduction

A stoma (plural stomata) is a pore, found in the leaf and stem epidermis that is used for gaseous exchange. The pore is bordered by a pair of specialized parenchyma cells known as guard cells that are responsible for regulating the size of the opening. They are usually kidney or bean shaped in dicotyledons. The cells surrounding the guard cells are known as subsidiary cells. Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stomata being counted as one cell. Salisbury[1] introduced the concept of stomatal index (SI). The stomatal index is usually calculated using the formula of Cutter[2].

The present study is aimed to find stomatal indices in selected dicotyledonous and monocotyledonous plants. The selection of plants was made on the basis of evolution of dicot and monocot families. For convenience, only the plants belonging to extreme ends of evolution were selected. Families such as Magnoliaceae, Annonaceae, and Malvaceae represent a primitive line and Asteraceae, Rubiaceae etc represent an advanced line of dicotyledonous plants. A comparison of stomatal index of selected monocot members is also intended. Members of Amaryllidaceae, Commellinaceae and Arecaceae were included among the monocotyledonous plants.

Materials and Methods

The selection of families for the current study is based on the concept of evolution. Some families which are conventionally regarded as "primitive" include Magnoliaceae and Annonaceae. Some such as Malvaceae are considered 'intermediate'. While Asteraceae and Rubiaceae are considered as 'advanced'. Monocotyledonous plants are represented by Amaryllidaceae, Commelinaceae, and Arecaceae and the selection was random. The genera selected for the present study is based on availability in the locality and time. The following plants were selected for the study; *Michaelia champaca* L (Magnoliaceae), *Annona reticulata* L. (Annonaceae), *Hibiscus rosa-sinensis* L. (Malavaceae), *Hamelia patens* Jacq., *Chasalia curviflora* Wall. (Rubiaceae), *Bidens sulphureus* Cav., *Tridax procumbens* L. (Asteraceae), *Tradescantia spathacea* Swartz. (Commelinaceae), *Caryota urens* L. (Arecaceae), and *Crinum asiaticum* L.(Amaryllidaceae).

Leaves show more stomata on the lower epidermis. So the tissue selected for the present study is lower epidermis of the sample. Study on upper epidermis was not conducted. Plants were collected from places around Thalassery during 2012. Stomatal peels were prepared using manual method using forceps and blade. Chemical methods were

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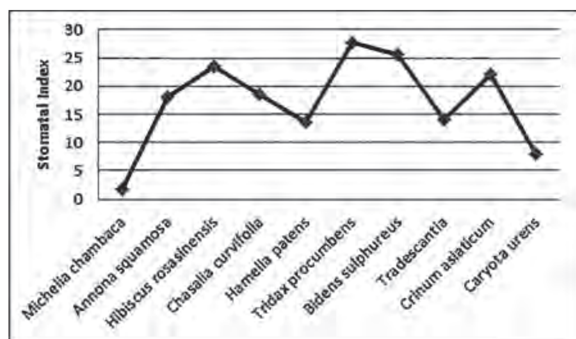

Fig. 1. Stomatal indices of selected plants

not employed to collect the peel. Peeled epidermis was stained with 4% Saffranin solution to stain the cellwall with pinkish color. Stained sample was mounted in glycerin and observed under Magnus MLX binocular microscope. The objective used is 40x except in the case of Rheo where 10x is used. Photographs of stained epidermal peels were taken using Olympus C-7070 digital camera.

Stomatal index was calculated using the equation of Cutter[2]. SI%= (S/E+S) x 100, where S= the

Table.1 Stomatal indices of selected plants.

Sl. No.	Name of Plants & Family	S	E+S	Stomatal index%	Average SI%
1	*Michelia champaca* (Magnoliaceae)	1	53	1.87	1.77%
		1	58	1.72	
		1	56	1.78	
2	*Annona squamosa* (Annonaceae)	60	341	17.59	18%
		54	288	18.75	
		58	328	17.68	
3	*Hibiscus rosa-sinensis* (Malvaceae)	15	55	27.27	23.45%
		12	52	23.07	
		10	50	20	
4	*Chasalia curviflora* (Rubiaceae)	9	51	17.64	18.61%
		11	58	18.96	
		10	52	19.23	
5	*Hamelia patens* (Rubiaceae)	9	73	12.32	13.54%
		16	94	17.02	
		17	151	11.25	
6	*Bidens sulphureus* (Astereceae)	32	128	25	25.25%
		31	123	25.2	
		35	137	25.54	
7	*Tridax procumbens* (Astereceae)	26	91	28.57	27.74%
		22	82	26.87	
		30	108	27.78	
8	*Crinum asiaticum* (Amaryllidaceae)	14	60	23.33	22.06%
		12	57	21.05	
		12	55	21.81	
9	*Tradescantia spathacea* (Commelinaceae)	1	10	10	14%
		2	10	20	
		1	8	12	
10	*Caryota urens* (Arecaceae)	11	128	8.59	7.91%
		8	123	6.5	
		11	127	8.68	

Table 2. A comparison of stomatal index with time of evolution of the selected plants

Sl. No.	Species and Family	Stomatal Index	Time of Evolution (million years)
1	*Michelia champaca*(Magnoliaceae)	01.77	127
2	*Annona squamosa* (Annonaceae)	18.00	110-106
3	*Hibiscus rosa-sinensis* (Malvaceae)	23.45	68
4	*Chasalia curviflora* (Rubiaceae)	18.61	78
5	*Hamelia patens* (Rubiaceae)	13.54	78
6	*Bidens sulphureus* (Astereceae)	25.25	42-36
7	*Tridax procumbens* (Astereceae)	27.74	42-36
8	*Crinum asiaticum* (Amaryllidaceae)	22.06	91
9	*Tradescantia spathacea* (Commelinaceae)	14.00	89
10	*Caryota urens* (Arecaceae)	07.91	120

number of stomata, which was counted by observing the stomatal openings and E= the number of epidermal cells. The subsidiary cells were also counted among the epidermal cells.

Results and Discussion

Stomatal indices of plants were obtained, tabulated (Table-1) and a graph was plotted using Microsoft Excel 2007 (Fig.1). A comparison of stomatal index with evolutionary age of plants is shown in Table 2.

Michelia chambaca a member of Magnoliaceae showed the least stomatal index. Magnoliaceae is a family that evolved 127 million years back[3]. *Bidens sulphureus,* a member of Asteraceae, showed the highest value of stomatal index. Asteraceae evolved 36 million years ago[4]. Among the monocotyledons, *Caryota urens* (Arecaceae) showed the least number of stomata. Arecaceae dates back to 120 million years in evolutionary time scale[5]. The highest number among monocotyledons was shown in *Crinum asiaticum* (Amaryllidaceae). Amaryllidaceae evolved 91 million years back[5]. The study on stomatal indices of selected species based on evolutionary time scale, shows distinct trend in the number of stomata found on the lower epidermis of plants. The general trend based on the present study is, the number of stomata is found usually higher in most recently evolved families and genera. The increase in stomatal number in recently evolved families and species is an indication that a higher rate of gaseous exchange is correlated to the advancement of plant life.

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Cytotoxic and Genotoxic effects of the Pesticide Ekalux (EC_{50}) on the root meristems of *Allium cepa*

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Abstract

The cytotoxic and genotoxic effects of quinalphos based pesticide of Ekalux (EC_{50}) was studied using *Allium cepa* as a test model. The varying concentrations of Ekalux (EC_{50}) {(10%, 20%, 40%,60% and 80%)} were used for conducting the experiment. It was found that the Ekalux (EC_{50}) inhibited the growth of the root length of the onion roots which was dependent on the concentration. Cytotological assays showed a decrease in the mitotic index with increase in concentration of the Ekalux (EC_{50}). The presence of increased percentage of the chromosomally abberent cells with bridges, breaks and micronuclei showed its clastogenic effects and laggard chromosome indicated Ekalux (EC_{50}) to be a spindle poison.

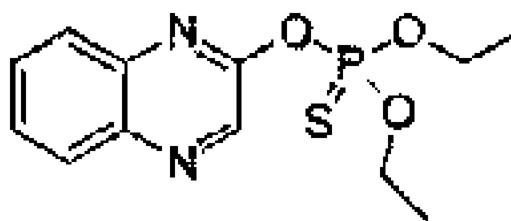
Key words: Ekalux, genotoxic, chromosome aberrant cells, mitotic index, *Allium cepa*

Introduction

One of the strategies employed to increase crop production is effective pest management. In tropical countries, crop loss is severe due to high temperature and prevalence and humidity, which is conducive for the rapid multiplication of the pest. Hence, application of wide variety of pesticides becomes a prerequisite to combat pest and vector borne diseases. The indiscriminate use of the pesticide in crop production justifies the evaluation of toxicity of various pesticides. The inappropriate application of pesticides makes them available in the environment, being globally and locally dangerous for the ecosystem and human population [1]. Such environmental pollution has increased tremendously for the last few decades due to anthropogenic activities. Agrochemicals necessary for crop production are significant pollution source. The chemical possess biological activities including genotoxic influence. Therefore, pesticides with mutagenic potential can influence non-target organism and affect human health adversely [2]

Ekalux (EC_{50}) is a pesticide which contains Quinalphos, a synthetic organophosphate, non-systemic, broad spectrum, insecticide and acaricide. It is highly active against biting and sucking insects and due to its short life with easy and detoxification in animal tissue, they are still widely used in agriculture.

Quinalphos chemical structure



IUPAC name

O,*O*-Diethyl *O*-2-quinoxalinyl phosphorothioate

Other names

O,*O*-diethyl *O*-quinoxalin-2-yl phosphorothioate; Diethquinalphion; Diethquinalphione

The *Allium cepa* test was chosen to determine genotoxicity due to its following advantages such as the root growth dynamic is very sensitive

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to pollutant, mitotic phase are very clear in onion, it has stable number of chromosome, diversity in chromosome morphology, stable karyotype, clear and fast response to genotoxic substance and spontaneous chromosomal damage occur rarely. Over the past decades, issues of animal use and case in toxicological research and testing have become one of the fundamental concerns for both science and ethics[3]. The principle of human animal experimentation —replacement, reduction and refinement (known as 3R'S) were defined by Russel and Burch. According to these principle alternative test object have been searched. Fiskerjo underlined that "A standard test for toxicity must be easy to perform and the result should be rapidly obtained and reproducible" [4]. Many plant species were tried out to provide valuable genetic assay system. Among the plant species, *Allium cepa* is considered to be a suitable test system for the evaluation of the genotoxic potential of pesticides [5,6]. According to FisKesjo, "results from Allium test have shown good agreement with results from other test system both eukaryotic as well as prokaryotic"[7]. The objective of the present study was to evaluate the cytotoxicity and genotoxicity of Ekalux (EC_{50}) using *Allium cepa* Assay.

Materials and Methods

Test organism

Healthy and equal sized bulbs of *Allium cepa* were chosen. Just before the experiments the outer scales of the onion bulbs and old roots were removed with the help of sharpen and pointed forceps so as to expose the root primordial. Care was taken not to destroy the root primordial. The experiments were maintained in laboratory conditions and roots were protected from direct sunlight in order to minimize fluctuation in the rate of cell division.

Test chemical

The test chemical namely the pesticide Ekalux (EC_{50}) , was purchased from Syngenta India Ltd., Pune , Maharastra , India

Test procedure[8]

Allium cepa root growth test

Clean and healthy onion bulbs (approximately about 100 numbers) were allowed to produce roots in coupling jars filled with distilled water, after 2 days the onion bulbs with freshly emerged roots were placed on coupling jars filled with different concentrations of Ekalux (EC_{50}) (Fig.1) . Some onion bulbs kept in distilled water served as control (Fig. 2)).The root length from control and experimental groups were measured (length of 10 roots from each bulb) (Fig.3). The relative reduction in the root length was calculated as percentage of deviation from the control. The effective concentration (EC_{50}) value was determined as the effective concentration for 50% growth inhibition.

Fig.1 Experimental Set up (Onion root tips imemersed in varying concentrations of Ekalux)

Fig.2 Coupling jar with onion bulbs in distilled water serving as control

Fig.4 Root tips stained in Acetocarmine, Toluidine Blue , dried and viewed under microscope

Fig.3 Measurement of Root length in onion bulbs in Root Growth Assay

Cytological Investigations[7, 9]

Fixation

After treatment the bulbs were washed thoroughly in running tap water and fixed in Clarke's fluid (Glacial acetic acid;95% Ethanol; 1:3) for 24hrs.

Squash Preparation

After fixation, the roots were hydrolyzed in 1 part of 1NHCl for 1 minute at 60°C and transferred to a watch glass containing aceto-orcein)/ Toludine Blue. They were then intermittently heated for 3-5min and covered and kept aside for staining for 20mins (Fig.4). The tip of the root was then cut with a sharp blade by placing on a glass slide. The root tip was then squeezed by tapping under the cover slip or with match stick and sealed with DPX or nail polish and examined microscopically.

Scoring of the slides

The slides were viewed under the light microscope (Labomed trinocular research microscope) using 40X and 100X objective with oil immersion, a total minimum of 100 cells were scored in each slide. The cells were recorded as normal or abberate in different stages of cell cycle. All cell abberations were counted and most respective one of each abnormality was photographed.

Data analysis

Cytotoxicity Assesment

The mitotic index was calculated using the method:

Mitotic Index= Total number of dividing cells/ Total number of cells counted X 100

Any dose of the test chemical was adjudged to be cytotoxic if the mitotic index of treated cells at that concentration was half or less compared to mitotic index of concurrent distilled water treated cells.

Genotoxicity assessment

The dividing cell with any of the underlisted chromosomal aberration /abnormalities was recorded a) chromosome fragmented (b) chromosome bridges (c) Abnormal metaphase (d) Lagging chromosome. (e) micronuclei/binuclei etc...

The number of abberated cells / 100 cells in each of the forms of division stages for of Ekalux (EC_{50}) treated cells were compared with number of water treated cells.

Statistical analysis

The control and experimental data were represented as Mean + SD in triplicates and data was processed by student's't' test

Table 1. Table showing the effect of Ekalux (EC_{50}) pesticide (at varying concentration in % solutions) on the root length (cms) of *Allium cepa* at an exposure period of 48 hrs.

Sl. No.	Groups	Root Length (cms.)
1.	Control	2.9±0.2
2.	Experimental	
	10%	1.6±0.4*
	20%	1.3±0.3*
	40%	1.0±0.6
	60%	0.7±0.6*
	80%	0.2±0.2*

*p value significant at 0.05 using student's 't' test

Fig. 5 *Allium Cepa* Test in control group. Note the Root length in Cms.

Fig. 6 *Allium Cepa* Test in experimental group treated with Ekalux (EC_{50}). Note inhibition of root growth.

Results and Discussion

Root Growth Assay

The effect of varying concentrations of Ekalux (EC_{50}), on longitudinal growth roots was analysed (Table.1 & Fig.5, 6, 7) at 48 hrs. At all concentrations of experimental groups, the root growth was

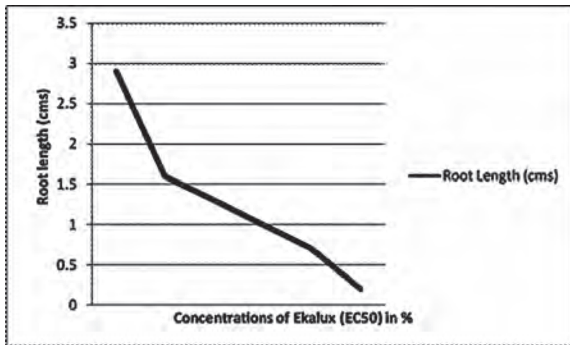

Fig.7 Line Diagram showing the effect of Ekalux (EC_{50}) pesticide (at varying concentration in % solutions) on the root length (cms) of *Allium cepa*

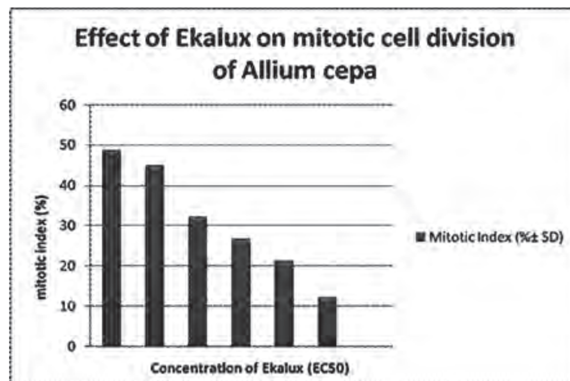

Fig.8 . Bar Diagram showing the effect of varying concentrations of Ekalux (EC_{50}) on mitotic index of *Allium cepa* at 48 hrs

Table 2. Table showing the effect of varying concentrations of Ekalux (EC_{50}) on mitotic cell divisions of *Allium cepa* at 48 hrs

Groups	No. of cells observed	No. of dividing cells	Mitotic Index (%± SD)
Control	270	131	48.6±0.2
Experimental			
10%	310	183	44.8±0.2*
20%	252	81	32.2±0.3*
40%	240	64	26.6±0.4
60%	170	36	21.2±0.2*
80%	210	26	12.1±0.6*

*p value significant at 0.05 using student`s ‘ t ‘ test

Table 3. Results of genotoxicity testing of varying concentrations of Ekalux (EC_{50}) in *Allium cepa* root assay

Groups	No. of cells observed	Chromosomal Abberations					Total Abberations (% ±SD)
		Da	Lg	Bg	Bk	Mc	
Control	53	1	-	-	-	-	1.8±0.23
Experimental							
10%	42	2	2	1	1	0	14.28±0.44
20%	56	2	1	2	1	0	10.71±0.32
40%	40	-	1	1	2	2	12 .5±0.12
60%	43	1	1	2	3	3	20 ± 0.41
80%	35	1	1	4	3	2	30.5 ± 0.32

Note: Bg – Chromatin bridge ; Da – Delayed anaphase ; Lg – Laggard chromosome ; Mc – Micronuclei ; Bk – Chromosome breaks

reduced compared to the control group (Fig.5,6). These findings indicate that Ekalux (EC_{50}), caused an inhibition of root growth in a concentration dependent manner. No complete inhibition was observed in any of the Ekalux (EC_{50}) exposed groups.

Effect on Mitotic Index

The results revealed that there was a decrease in the Mitotic Index (MI) of Ekalux (EC_{50}) treated roots and the decrease was significantly dependent on the concentration (Table 2.). The Fig. 8 shows a graph plotted between mitotic index of the Ekalux (EC_{50}) exposed roots at different concentrations .

Chromosomal Abberations

Results of the microscopic analysis of Ekalux (EC_{50}) treated root tips of *Allium cepa* is summarized in Table 3 . Fig.9 a-e shows the chromosomal aberrations which were induced at all concentrations of Ekalux (EC_{50}) exposed groups and were statistically significant. The various kinds of chromosomal aberrations, such as a) chromosome fragmented (b) chromosome bridges (c) Abnormal metaphase (d) Lagging chromosome. (e) micronuclei/binuclei etc were recorded indicating the genotoxicity of Ekalux (EC_{50}) pesticide. Chromosomes with disturbed spindles and fragments and bridging of the chromosomes were quite common. Vacuolization of the cytoplasm and rupture of cell membranes were seen in any cells following treatment at higher concentrations.

Our findings suggest that Ekalux (EC_{50}) inhibits longitudinal growth of the roots and the inhibition was dose dependent as there was a delay in the growth of the roots. With increase in the concentration of the Ekalux (EC_{50}) , the growth rate of the tips decreased significantly. This probably indicates the cytotoxicity of the pesticide Ekalux (EC_{50}) where the cell divisions of the meristamatic cells of the root tips was slowed down . Ekalux (EC_{50}) also decreased the mitotic index in *Allium cepa* root tip cells. The decrease in mitotic index was significant in almost all concentrations of Ekalux (EC_{50}) treated groups. The possible mechanism capable of bringing about decrease in mitotic index is a. by blocking of G1 phase suppressing DNA synthesis b. by blocking G2 phase , preventing cells from entering into mitosis 10. The chromosomal aberrations induced by

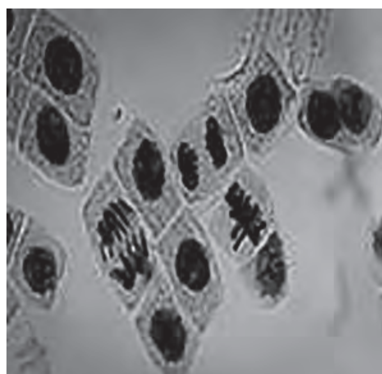

Fig.9 Control root tip cells

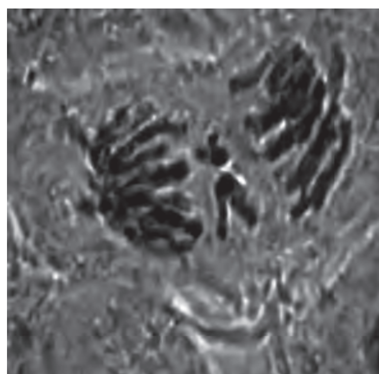

Fig.9a Anaphase with laggard chromosome

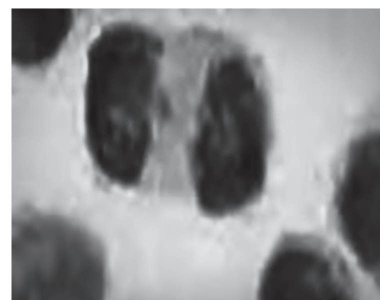

Fig.9b Anaphase lag

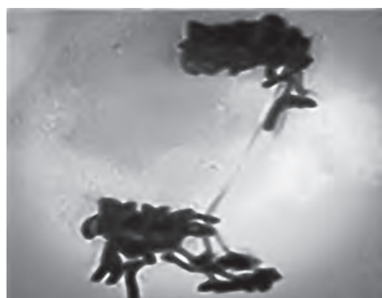

Fig.9c chromosome bridge with breaks

Fig.9d Micronuclei

Fig.9e Chromosome bridge with fragments

Fig.9 Various chromosomal aberrations in comparison to control on exposure to Ekalux (EC_{50})

Ekalux (EC_{50}) were more common at metaphase and anaphase stages indicating its genotoxicity.

The effects of chromosome bridges, breaks / fragments was indicative of chromatin dysfunction, laggard chromosome was indicative of spindle failure. The defects as chromosome breaks, bridges and laggardness are indicative of increased risk for aneuploidy 1 . Similar results have reported with various other pesticides as benomyl 11, Acetamiprid 12 , Dithane, Malthion and Garden ripcord 5, 13 , diphenyl-ether herbicide GOAL(Oxyfluorfen) 14 , Neemstra 15. The abnormalities as lagging chromosome and fragments were reported to be due to the disturbances in the spindle fibre formation 10. The anaphase bridges observed in the present study might be due to formation of dicentric chromosomes as a result of breakage and reunion of broken chromosomes. It is also of the opinion that anaphase bridges might be due to unequal exchange of dicentric chromosomes 16.Micronuclei often results from acentric fragments or lagging chromosomes that fail to incorporate into daughter nuclei during telophase of mitotic cell and cause death due to deletion of certain genes 16. The presence of micronuclei is considered as indication of true genotoxic effect. The results of the present study show a significant reduction in the mitotic index indicative of the mitodepressive effect. Since the pesticide is causing abnormalities on the cell division process leading to genetic damage, it has the potential to cause adverse effects to both human and environment, hence judicious use of pesticides is essential.

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An *In vitro* study on the efficacy of commercially available mouthwashes on oral bacterial load in healthy individuals

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Abstract

Increasing number of people are using mouthwashes for general and oral health care. Mouthwashes also provide a safe, effective chemical means of reducing or eliminating plaque accumulation. The aim of this study was to determine the antimicrobial properties of commonly available mouthwashes against oral pathogens related to caries and to oral infections, to verify the claims made by the manufacturers to provide information to dental professionals about the efficacy of their products *in vitro* and to use these mouthwashes as a base for the evaluation of antimicrobial plant products. In the present experimental study, two different techniques were used for analysis, Agar well diffusion method to determine the zone of inhibition in mm and contact test method to determine the bacterial count *in vitro*. Significant difference ($P < 0.05$) was observed among various mouthwashes when the mean diameter of inhibition zone was compared. The control microbial load showed a preponderance of *Staphylococcus aureus,Streptococcus pyogenes,Streptococcus mutans, Helicobacter pylori,Actinomyces viscosus, Candida albicans* and *Porphyromnas gingivalis.* All the mouthwashes were effective in reduction of the bacterial count from baseline to 24 h. In view of these findings, the present study indicates that these mouthwashes can be used as chemical plaque control agent to prevent oral disease and to improve oral hygiene.

Key words: Mouthwashes, Antimicrobial property, Agar well diffusion method, *Staphylococcus aureus*, Oral hygiene

Introduction

Poor oral hygiene is seemingly a common problem encountered by restorative dentists with their numerous dental patients. Poor oral hygiene precipitates tissue responses which are direct sequels to accumulation of food debris, tartar, stains and proliferation of microorganisms[1]. Other distant infections such as pulmonary infections and infections of the gastrointestinal tract have also been observed[2]. Mouthwashes have been particularly well-accepted by individuals due to their ease of use[3].

Mouthwashes (Mouthrinses) are solutions or liquids used to rinse the mouth for a number of purposes: (a) to remove or destroy bacteria (b) to act as an astringent (c) to deodorise and (d) to have a therapeutic effect by relieving infection or preventing dental caries. Constituents of mouthwashes include water (as chief constitutent); ethanol, dyes, surface active agents, zinc chloride/acetate, aluminium potassium sulphate (as astringent); and phenolic compounds, quaternary ammonium compounds and essental oils such as oil of peppermint (as antibacterial agents) among others[4, 5, 6]. A number of chemical agents are currently available in the market and are designed to assist individuals in their efforts to achieve and maintain oral health. While many agents are commercially available, the relative therapeutic benefits of most are not clearly defined. Kornman (1986) has suggested an organizational frame-work which allows classification of these topical anti-microbial into one of two categories or generations based on their pharmacological properties[7]. First generation agents can kill bacteria on contact, but have limited abilities to exert an effect on the oral flora after expectoration (e.g. cetylpyridinium chloride and sanguinarine), second generation agents have an immediate antibacterial effect and more importantly, have a prolonged

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effect on the oral flora (e.g. chlorhexidine). Mouthwashes are manufactured in two forms: the wash and the 'spray'. For most individuals the wash is a simple and acceptable method for the delivery of topical medicaments into the oral cavity. Rinsing with a chlorhexidine mouthwash is arguably the most effective chemical method to date of controlling plaque accumulation[8]. Based on the original study, the most common regimen of use has been twice daily rinsing with 10ml of a 0.2% chlorhexidine solution[4, 8, 9]. However, with the availability of more commercial mouthwashes[10, 11], similar antiplaque effects have been reported[12, 13] with twice daily rinsing with 15ml or 10ml of the solution according to manufacturer's direction of use. Some studies have shown the ability of mouthwashes on plaque accumulation, plaque composition, either biochemical or microbiological, the possible effect of a mouthwash on bacterial load count in the mouth has received little or no attention in human studies in this environment. Based on this scanty information, the present study was designed to investigate the ***in vitro*** effectiveness of some commercially available mouthwashes on oral bacterial load count in healthy individuals.

Materials and Methods

Subjects

The study population composed of 45 individuals (30 male and 15 female). All the individuals who agreed to participate in the study, (a) had to sign an informed consent, (b) must have full complement of the teeth; (c) must have a high standard of oral hygiene and gingival health, (d) had no relevant medical history, (e) were not receiving pharmacotherapy which might influence plaque accumulation or professional removal of plaque deposits and (f) were within (24-30) years old. Grounds for exclusion criteria included pregnancy, debilitating disease, the need for antibiotic cover for dental treatment, steroid therapy or antibiotic medication either current or during the previous eight weeks.

Subject grouping and Saliva Sampling

All the volunteers were divided into three groups: 15 subjects per group, each group having 10 males and 5 females. Spitting method was employed in which the subjects were seated with head inclined forward so that the saliva will collect in the floor of the mouth from where it is spat into a 20ml sterile specimen bottle for 5 minutes. The saliva samples collected in the first day for each group served as the control and the samples collected after 8 weeks of daily use of the grouped mouth rinses served as the experimental.

Subjects' saliva was collected early in the morning after normal oral hygienic procedures. Subjects were asked to brush with the particular paste they were using before the start of the experiment. In each group, subjects rinsed the mouth first with 40ml water for 10 seconds after normal oral hygiene procedure (tooth brushing with toothpastes). Saliva sample were collected by a single investigator and transferred directly to the laboratory for analysis. The samples were then cultured for bacterial colonies under standard incubatory conditions on blood and chocolate agar, which are the most common culture media used for the culture of oropharyngeal bacteria. Standardized streaking techniques were followed using a sterile loop of known volume (1/500 ml).

Mouth rinses

The products evaluated in this study were Hexidine®, Colgate Plax® and Listerine®.

Estimation of Bacterial Counts

The colonies were counted after 48 h incubation. The total numbers of bacterial colonies were counted and multiplied using a factor based on the volume of the streaking loop. This procedure was standardized using quality control measures and followed on all the culture plates[14, 15]. The scoring pattern used in this study were, score 0 resembled No growth, score I resembled <1000 colony-forming units (CFU), score II resembled 1000-10,000 CFU, score III resembled 10,000- 100,000 CFU and score IV resembled >100,000 CFU respectively.

Statistical Analysis

The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. The significance of the differences between control and experimental were analyzed using Student's t-test. The experiments were performed in duplicate and the mean values of the diameter of inhibition zones with ± standard deviations were calculated. The duplicate plate counts derived from each sample were converted to CFU/ml. The sample CFU scores were logarithmically (base 10) transformed in order to obtain

normal distribution. One way analysis of variance (ANOVA) was performed for determining if significant differences existed between the reductions in CFU for the different agents. Statistical Package for Social Sciences (SPSS version 17, SPSS Inc., Chicago) was used for analysis. $P < 0.05$ was taken as statistically significant.

The results of the present study showed that, when the mean number of bacterial colonies at full strength (1:100) of concentration were compared, there was significant difference ($P < 0.05$) observed between mouthwashes (Table 1, 2 and 3). All the three mouthwashes showed excellent antimicrobial activities.

Table 1. Mean bacterial load count in healthy adults using Hexidine® mouthrinse.

Saliva Sample	Control		Experiment	
	Male	Female	Male	Female
Microbial Count (CFU/ml)	$9.06x10^8 \pm 0.5x10^2$	$7.43x10^8 \pm 0.3x10^2$	$5.21x10^8 \pm 0.4x10^{2*}$	$3.87x10^8 \pm 0.1x10^{2*}$

Note: *Significantly different, P < 0.05, CFU/ml- colony forming units

Table 2. Mean bacterial load count in healthy adults using Colgate Plax® mouthrinse

Saliva Sample	Control		Experimental	
	Male	Female	Male	Female
Microbial Count (CFU/ml)	$8.21x10^8 \pm 0.8x10^2$	$7.18x10^8 \pm 0.3x10^2$	$6.42x10^8 \pm 0.2x10^{2*}$	$4.64x10^8 \pm 0.2x10^{2*}$

Note: *Significantly different, P < 0.05, CFU/ml- colony forming units

Table 3. Mean bacterial load count in healthy adults using Listerine® mouthrinse

Saliva Sample	Control		Experimental	
	Male	Female	Male	Female
Microbial Count (CFU/ml)	$9.21x10^8 \pm 0.7x10^2$	$8.02x10^8 \pm 0.5x10^2$	$7.13x10^8 \pm 0.4x10^{2*}$	$6.21x10^8 \pm 0.3x10^{2*}$

Note: *Significantly different, P < 0.05, CFU/ml- colony forming units

Results and Discussion

A total of forty five subjects (30 male and 15 female of age range 24 to 30 years) were recruited into the study and received the baseline examination. None was excluded and all groups were well-matched at the beginning of the study with respect to age, sex, number of teeth, clinical condition and compliance with the rinsing programme. The subjects cooperated well, and none had any complaint throughout the study. The efficacy of three antimicrobial mouthrinses (Hexidine®, Colgate Plax® and Listerine®) was evaluated after 8 weeks of continous daily use. The control microbial load showed a preponderance of *Staphylococcus aureus, Streptococcus pyogenes,Streptococcus mutans, Helicobacter pylori,Actinomyces viscosus, Candida albicans* and *Porphyromnas gingivalis.*

Of all the three, Hexidine® mouthwash was found to be the most effective (Table.1; Fig.1). Chlorhexidine- based formulas are currently the golden standard for antimicrobial mouthwashes. Chlorhexidine gluconate is a cationic biguanide with broad spectrum of antimicrobial action. Its mechanism of action is that the cationic molecule binds to the negatively-charged cell walls of the microbes, destabilizing their osmotic balance causing concentration-dependant growth inhibition, and cell death[16]. Secondary interactions causing inhibition of proteolytic and glycosidic enzymes may also be significant[17].

Relatively recent information on the literature regarding triclosan[18]; however has generated interest based not only on its antimicrobial and anti-inflammatory activity, but especially because of the absence of undesirable side effects. Triclosan

is a nonionic phenol having broad spectrum of antimicrobial activity with moderate substantivity. It has been formulated with a copolymer to improve its substantivity and 0.03% triclosan mouthwash achieves moderate reduction in plaque. Listerine® mouthwash, is a combination of essential oils (eucalyptol, menthol, thymol and methyl salicylate), which has been proved efficacious for the reduction of plaque and gingivitis[19].

It is known that a balance exists in a person's oral microbial population. If this balance is lost, opportunistic microorganisms can proliferate, enabling the initiation of disease processes. Therefore, the mouthwash identified as having the largest micro-

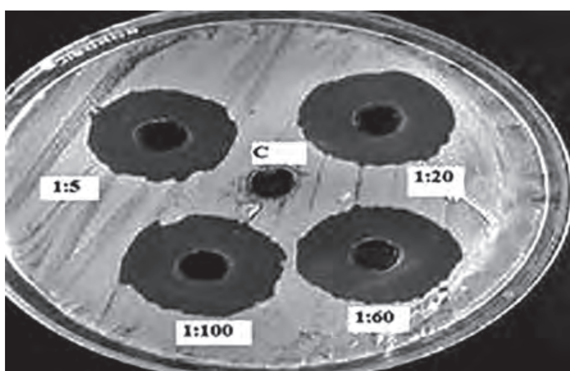

Fig. 1. Zones of inhibition produced by Hexidine® mouthrinse (24 h) at different concentrations, C- Control

bial inhibition zone-and thus probably the strongest antibacterial and antifungal properties-may not be necessarily superior to those found to have smaller diameter inhibition zones. Because a mouthwash used *in vivo* likely is diluted by saliva, the level to which antimicrobial properties are buffered or lost in dilution *in vitro* is of interest[20]. In addition, dentists should keep in mind that the mean average inhibition zone of one mouthwash may not be directly comparable with that of another mouthwash because different mouthwashes are constituted of different active ingredients and may diffuse at different rates. It is important to bear in mind that an experiment conducted *in vitro* has limitations, as it is considered a static system compared to *in vivo* tests, which may reflect the influence of various dynamic factors like systemic conditions, salivary flow, diet and dental anatomy. Nevertheless, it might be considered that if the antimicrobial agent does not have activity *in vitro* it most likely will not work *in vivo*. In this sense, assessments of antimicrobial activity conducted using cultures *in vitro* enable a direct contact between the bacterial colonies and the chemical substances tested for a period of 28-48 h.

In conclusion, the results of the present study showed that when used unsupervised as a part of regular oral hygiene and professional care; chlorhexidine, phenolic compounds or glycerin/triclosan containing mouthwashes provide significantly greater oral microbial reductions. However, where results are statistically significant, consideration should always be given to their clinical efficacy. We also conclude that with the unaffordable price hikes and the introduction of new brands of mouthwashes with improved formulations, it is up to the consumer to decide what he or she rates most highly in a mouthwash: breath freshness, oral cleansing or an antiseptic action to control oral microorganisms and/ or pathogens and to choose accordingly. Nevertheless, extensive laboratorial studies are needed to explore the performance of further clinical investigations.

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Studies on the host preference and oviposition decisions in the pulse beetle, *Callosobruchus chinenesis*

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Abstract

Callosobruchus chinenesis is a Bruchid that cause economic loss by damaging the stored grain pulses. The females lay eggs on the pulses, and the hatch out larve feeds on the entire content of the pulses. The present study was proposed to demonstrate the selection or preference of host from different pulses for oviposition. The experimental infestation of different stored grains under laboratory conditions showed that the red gram was mostly preferred by *C.chinensis* for oviposition and the oviposition decisions involving discrimination among the different pulses is based on the resource quality.

Key words: *Callosobruchus chinenesis*, oviposition decision,

Introduction

Bruchids are a group of beetles that cause economic loss by damaging the stored grain pulses. The group mainly include *Callosobruchus chinenesis*, *C. maculates* and *C. analis*. The adults are small brown beetles with light bands and markings on their backs. The larvae, which are responsible for the major damage, are small, white, C- shaped worms with darker heads. The hatching larva immediately bore inside and larval period, feeding within the seed. The life cycle can be completed in about one month or less and continual generations are possible until the food source is exhausted. The infestation may originate in the field and continue in storage.

C. chinensis is cosmopolitan pest of stored legumes. Females cement eggs to the surface of the host seeds[1]. Larvae burrow into the seeds where their entire development (four instars and pupal stadium) is completed. Larvae cannot move among seeds and are thus restricted to the seed that their mother has chosen for them. Beetles emerge from seeds reproductively mature. Emerging adults are well adapted to storage conditions, requiring neither food nor water to reproduce, because beetles most commonly occur in seed stores.

The ability of parasitic insects to discriminate between hosts of differing quality can substantially affect their lifetime fitness. Hosts of many parasitic insects (such as parasitoids and seed beetles) are discrete and limited in size and thus vary in their quality for parasite development and fitness [2]. Oviposition decisions should be based in part on resource quality with females preferring higher quality resources[3]. When hosts are limiting, females must also choose between laying either clutches of eggs, laying additional eggs on previously parasitized hosts or laying fewer eggs.

Optimality models predicts that females should lay smaller clutches and be less willing to super parasitize on lower quality and/ or smaller hosts because larval competition increases and progeny fitness decreases with decreasing host size,[2,4,5,6]. These predictions are generally consistent with findings in parasitic insects- females of most species prefer to oviposit on larger, higher quality hosts and generally lay smaller lay smaller clutches on smaller and lower quality hosts [7, 8, 9, 10].

The present study was proposed to demonstrate the selection or preference of host by the pulse beetle *Callosobruchus chinensis* on the availability of single and mixed grains as hosts, the fecundity studies of pulse beetle C.chinensis influenced by the stress generated by changing the host seeds and also the influence of surface area of the seeds on oviposition descisions.

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Materials and methods

Collection of the beetles

The beetles used for our experiments were collected from infested red gram pods obtained from the local market and store houses.

Selection of grains

For the present study, different stored grains such as *Cicer arietinum* (Bengal gram) *Vigna mungo* (Black gram), *Phaseolus radiates* (Green gram), *Dolichos biflorus* (horse gram) , *Zea mays* (Maize) and *Triticum vulgare* (Wheat) were selected.

General experimental procedure

The selected grains were fumigated with aluminium phosphide and kept for five days before experimental infestation. Then the newly emerged insects were introduced into the grains in separate containers. All seeds were checked every 24 hr. for emerging adults and the females were allowed to mate. Thus, all females were 24hr old at the initiation of each experiment. Then they were confined in a 35mm petridish with seeds of different grains and allowed to lay eggs. The number and size of seeds presented, and the time that females were allowed to oviposit, varied among experiments.

Experiment 1

In our first experiment, we observed the rate of oviposition, time period and delay in oviposition and fecundity with respect to the seed type in separate containers.

Experiment 2

In the second experiment we hypothesized (a) the selection or preference of seeds by the female for oviposition and when encountering different seeds of similar size, females distribute their eggs relatively uniformly among them ; and (b) females have higher realized fecundity when encountering large seeds, indicating that they superparasitize more readily on large seeds than on small seeds. Females were briefly distributed at 24 hr. to count the number of eggs laid on each seed; eggs were counted with a hand lens.

Experiment 3

This experiment was designed to test whether females distributed their eggs among seeds consistent with predictions of our simple resource model of experiment 2. Different types of the selected seeds were sorted as in experiment 1. Females were then allowed to lay eggs for 48 hr and eggs were counted periodically at 12, 24 and 48 hr. Females in the predicted proportion of eggs that females should lay on preferential seeds, was calculated separately for each seed type. The actual proportion of eggs that females laid on large seeds was calculated. We estimated the proportion of eggs that females laid on large seeds if they use surface area to measure size.

Results and Discussion

The experimental infestation of different stored grains under laboratory conditions showed that the red gram was mostly preferred by *C. chinensis* for oviposition. The percentage infestation showed that the highest rate of infestation is in the red gram (13.7%). It is also observed that, the highest number of egg mass is seen on the surface of red gram itself. At the same time, least prefererence for egg laying was on wheat on which no oviposition was

Table 1. Oviposition decisions of *C.chinensis on* diferrent seed samples

Grain	Total No. Observed	Oviposition observed	% oviposition per seed	Highest no. of oviposition per seed	Lowest no. Of oviposition per seed	No. Of emergence of insects
Red gram	40	40	13.7	26	5	15
Green gram	40	40	3.5	6	2	32
Black gram	40	40	5.3	9	1	11
Horse gram	40	39	2.6	7	1	19
Maize	40	26	1.3	4	1	0
Wheat	40	0	0	0	0	0

observed. A highest percentage emergence of adult was observed from the green gram (32)(Table 1).

In the third experiment we observed the red gram was with the highest rate of ovoposition (14.2) per seed. Here also the wheat was not selected by insects to deposit their eggs. The data collected on the size, shape and other features also of the grains for analysing role of these factors on oviposition.

In the present study we examined egg-laying decisions by the pulse beetle, Callosobruchus chinensis (Coleoptera: bruchidae) to test whether they conform to predictions of an optimality model. The observations showed that the ability of females to accurately access the number of eggs laid on a seed. The present findings are in a agreement with seed will provide her progeny with twise as much biomass as the smaller seed, and so she should lay her egg on the larger seed, Mitchell 3 But Avidov et al.12 suggested that females prefer only smaller seeds with high resource quality. If two eggs have already been laid on the larger seed, and none on the smaller seed, then the smaller seed will provide more resources, and should thus be preferred. If there are two eggs on the larger seed. As egg density increases females should lay on average, twice as many eggs on the larger seed as on the smaller seed.

Our data suggested that the female beetles evaluate the relative quality of resources available inside of a seed more accurately than if they compared the ratio of surface areas between seeds of varying sizes. This is in agreement with the studies of Mitchell 3.

Table 2. The host preference status of *C.chinensis*

Grain	Total.no. observed	Oviposition observed	% oviposition per seed	Length of grain	Breadth of grains	Weight of grains
Red gram	5	5	14.2	0.76	0.66	0.28
Green gram	5	5	3.8	0.34	0.22	0.03
Black gram	5	5	5.6	0.52	0.32	0.04
Horse gram	5	5	2.6	0.56	0.38	0.03
Maize	5	4	1.4	1.1	0.86	0.35
Wheat	5	0	0	0.78	0.3	0.04

the findings as, when hosts are superabundant, females usually distribute their eggs uniformly (n1 egg/seed) although the degree of uniformity varies among populations, Messina 1;Horng,11 However, when seeds are limiting most female *C.maculatus* readily superparsitize[3] .

Because seeds vary widely in size, these seed beetles provide an excellent system to test predictions concerning female oviposition decisions involving discrimination of resource quality and superparasitism. Seed size on oviposition decisions has not been well examined.

In this study, we compared egg- laying decisions made by female C.chinensis and the preference of host seed from the mixture of different seeds. This experiment demonstrated that there is some significance in resource quality that favours repeated use of a discrete, higher quality resource over the use of a discrete, lesser quality resource. A study reported that a female encounters two seeds, one of size smaller and other of larger: because the larger

Avidov *et al* .[12] proposed that females identify seed size according to surface curvature. Wilson (1988)[13] documented that female *C.maculates* spend over a quarter (27%) of their ovipositional time inspecting the seeds but, other than oviposition pheromones and species – specific host plant cues, it is unclear what information females acquire during these short span of inspection and how this information is processed by the female central nervous system.

Most research on optimal oviposition strategies of seed beetles has focused on the frequency of host encounters and the avoidance of superparasitism; the role of seed size is largely unconsidered. For instance, Mitchell and Thanthianga(1990)[14] list three reasons that *C.maculatus* oviposition behaviour deviates from randomness, none of which include variation in seed size. In our study, we found that not only seed size important during oviposition but that it directs oviposition behaviour away from a strict uniform dispersion. We

have shown that variation in seed size is important during all oviposition periods and at varying egg loads increase.

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A brief and precise literature review with objectives of the research under taken and essential background be given.

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