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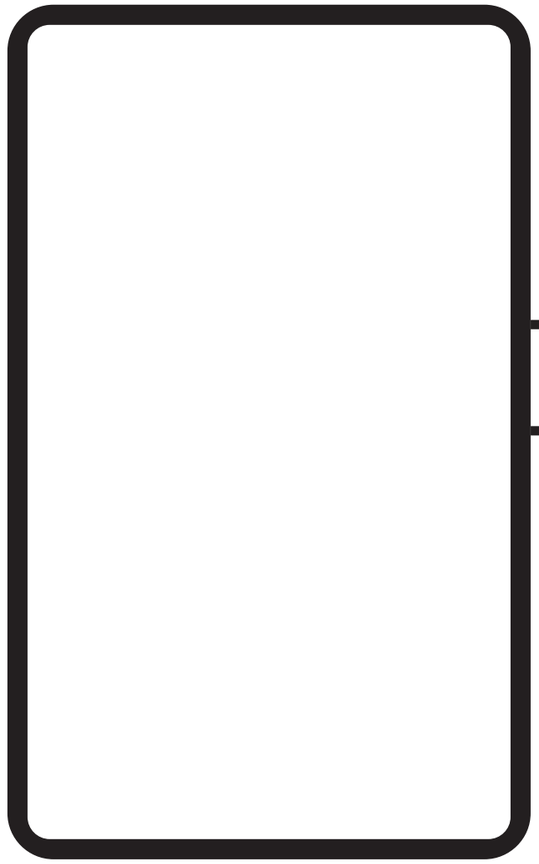

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FROM THE DESK OF
GENERAL SECRETARY, AZRA

I am pleased to inform all the members of AZRA that a few important decisions have been taken this year during 21st AZRA General Body Meeting held on 3 August, 2018 at ICAR-Central Institute for Women in Agriculture (CIWA), Bhubaneswar for the better functioning of the Association. These are:

a. Henceforth, with every General Body Meeting of AZRA, an invited Guest Lecture will be arranged on the suitable theme/topic related to applied zoology. To start with, an invited First Guest Lecture was given by Dr. J. K. Sundaray, Former Director, CIFA, Bhubaneswar, during 21st AZRA AGM on 3rd August, 2018 at ICAR-CIWA, Bhubaneswar on "**Aquaculture for augmenting food & nutritional security**", which was attended about 50 members and invitees. This meeting was organized by Dr. S. K. Srivastava, Director, CIWA, as AZRA Executive Council Member.

b. It was decided to rename the AZRA Award- "Prof. P. Kameswara Rao Award for Best Poster Presentation" during AZRA Conferences as "**Prof. S. K. Shrivastava Award for Best Poster Presentation**", keeping intact "**Prof. P. Kameswara Rao Award for Best Oral Presentation**".

c. It was also approved to institute an award for one best research paper published in Journal of Applied Zoological Researches (during a lot of two years issues) initiated from 2018 & 2019 and this award is named as "**Prof. Damodar Satapathy Award**". This award will be conferred during next AZRA Conference scheduled in February, 2020 at University of Agricultural Sciences (UAS), Raichur, Karnataka (dates to be announced shortly). Thus persons submitting research papers of Journal of Applied Zoological Researches also have one more opportunity to recognition of their research work.

Through this letter, I heartly convey my **Best Wishes for a Very Happy, Healthy and Prosperous New Year- 2019** to all the members of AZRA.

Anand Prakash
Founder & General Secretary,
AZRA, Bhubaneswar

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INSECT AS BIOINDICATOR: AN UNTAPPED TREASURE

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ABSTRACT: Environmental pollution constitutes a serious threat to the existence of ecosystems, hence environmental monitoring becomes a constant element for its management and prediction. Class Insecta has many potential representatives that can be used as environmental bioindicators as they have a higher level of structural and functional organization, and have more complex morphology, physiology, more developed sense organs, complex behavior and are characterized by a greater diversity of species, therefore, they can more precise, more rapid and more variable to reflect disturbance of their environment, a xenobiotics may penetrate into the organisms via air, water, soil, dust and food, through the skin, respiratory system and alimentary tract. The use of bioindicators is an innovative approach for assessing various types of environmental mismanagement, including pollution, soil health high input farming, inappropriate disposal of wastes, contamination, etc. Globally, insect role as bioindicator to human support policy decisions for conservation and to evaluate functional consequences of human disturbance on ecosystems are recognized and we are ignoring this in India, hence it is discussed.

Key worlds: Insect bioindicator, climatic change, demographic change, seasonal shift, ecosystem indicator.

Global warming could lead to a rise in the snowline and disappearance of many glaciers causing serious impacts on the populations relying on the seven main rivers in Asia that are fed by melt water from the Himalayas. Throughout Asia one billion people could thus face water shortage leading to drought and land degradation by the 2050s (CHRISTENSEN *et al.,* 2007). Organisms not only provide information about their habitats but its sensitivity to a particular attribute make inferences about that attribute. In other words, they are a surrogate for directly measuring abiotic features or other biota. Bioindicators are evaluated through presence/absence, conditions, relative abundance, reproductive success, community structure, community function, or any combination thereof (HELLAWELL *et al*., 1988). The term bioindicator species was coined by KOLKWITZ and MARSSON in 1908, regarding the impact of organic pollution (i.e. sewage) on aquatic organisms (ROSENBURG and RESH, 1996). According to KOVACS *et al*. (1992) communities are generally regarded as the most appropriate indicators for conservation biology. A bioindicator can be defined as ‘‘**a species or group of species that readily reflects the abiotic or biotic state of an environment, represents the impact of environmental changes on a habitat, community, or ecosystem, or is indicative of the diversity of a subset of taxa, or of the wholesale diversity, within an area**’’ (MCGEOCH,1998).

Scan of information on bioindicator has shown that there developed two concepts; one of population bioindicator developed in around 1942 and second biodiversity indicators during 1980. It makes a broad and intangible concept such as biodiversity or ecosystem monitoring manageable by breaking it down into a specific set of quantifiable indicators (NOSS, 1990). Inference through biological indicators replaces direct

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measurement when such measurements are not possible, too expensive/difficult, or too direct (LANDRES *et al*., 1988; CARO and OÍDOHERTY, 1999). Biological indicators are currently used and promoted by numerous conservation agencies as a means to tackle biological monitoring and assess human impacts, including the World Conservation Union-International Union for Conservation of Nature (IUCN), World Conservation Monitoring Centre- United Nations Environment Programme (UNEP); U.S. Environmental Protection Agency (USEPA), as well as the Nature Conservancy, World Wide Fund for Nature (WWF), Friends of the Earth (FOE), and Greenpeace (IUCN, 1989; USEPA, 2002a; UNEP, 2002).

Insects are the most abundant animals in almost all the ecosystems and can be used to evaluate the impact of environmental change in different ecosystems, as they are more severely and quickly affected than other taxa by changes in the landscape (DA ROACA *et al*., 2010). Class Insecta has many potential representatives that can be used as environmental bioindicators, among them are many species from the Orders Coleoptera, Diptera, Lepidoptera, Hymenoptera, Hemiptera, Isoptera and can be potential indicator for changes in microclimatic conditions, foraging activity and nesting locations, reduced food availability from the use of agrochemicals and interactions with other species (DE BRUYN, 1999) and provide information on anthropogenic stress of an ecosystem (ROSENBERG and RESH, 1993). Utilization of insect bioindicators would be an inexpensive method for monitoring pollution and for carrying out preliminary assessments of the water quality of inland ponds and lakes. This would avoid direct assessment of water quality involving expensive analytical methods, particularly at the preliminary stages. Integration of inexpensive biomonitoring methods with chemical-specific assessment methods would facilitate the restoration of the biological integrity and ecological health of freshwater bodies. In short, role of insect as bioindicator has been categorized based on their functions (Table-1) and discussed further.

Table-1: Insects as bioindicators in different indicator categories & alternative functions

Indicator category	**Alternative functions indicator used to**	
Environmental	Detect a change in environmental state	Monitor changes in environmental state
Ecological	Demonstrate the impact of a stressor on biota	Monitor longer term stressor-induced changes in biota
Biodiversity	Identify diversity of taxa in a specified area	Monitor changes in biodiversity

1. Habitat indicator species

Habitat indicators provide information about the **quality of their habitat** through their physical condition or presence/absence, thereby effectively functioning as biological litmus paper (HELLAWELL, 1986). The scientific community accepted the need to assess how changes in the physical environment materialize in the biotic community (PAOLETTI, 1999; USEPA, 2002a), as well as the concept that managing for the most sensitive taxa accounts for other less sensitive taxa (SIMBERLOFF, 1998). There is little controversy concerning their validity since they involve very little inference and have a long history of study in the fields of botany and environmental toxicology (LANDRES *et al*., 1998; PAOLETTI, 1999).

2. Population indicator species

Population indicator species are also selected based on their sensitivity to particular environmental attributes. An important distinction is that their **population trends are**

extrapolated to reflect those of similar species, rather than the condition of their environment (CARO and OÍDOHERTY, 1999). This guild-indicator approach is charged with over simplifying interspecific relationships since species are different in their response to habitat changes and mechanisms of population control, and the removal or decline of one species may actually benefit a similar species by freeing up limited resources (CARO and OÍDOHERTY, 1999).

3. Biodiversity indicator species

The most recent application of bioindicator theory developed from the concept of biological diversity (WILSON, 1988) and the Rio Earth Summit in 1992 (UNEP, 1992). There is much discussion in the scientific literature as to the validity of biodiversity indicators with studies that both support (CARROLL and PEARSON, 1998 and REYERS *et al*., 2000) and oppose this theory (FAITH and WALKER, 1996, and VAN JAARSVELD *et al.,* 1998). Biodiversity indicators have been used to infer lower taxon (i.e. species) richness by surveying higher taxonomic levels (i.e. family) (supported by OLIVER and BEATTIE, 1993; GASTON, 2000; and disputed by GOLDSTEIN, 1997; PRENDERGAST and EVERSHAM, 1997), however they are more commonly used to identify **hubs of biodiversity by inferring overall diversity** from that of an indicator taxa (MCGEOCH, 1998; CARO and OÍDOHERTY, 1999).

Critique of biological indicator theory: The biological indicators have high intuitive appeal and have been eagerly embraced by many conservation managers, without regarding for selection criteria or empirical evidence (MCGEOCH and CHOWN, 1998). The most common criticism of indicator species is that scientifically invalid criteria have been used for their selection, whether this is socio-economic pressure to study charismatic macro-fauna (HILTY and MERENLENDER, 2000), or the desire to study convenient or favourite taxa (MCGEOCH, 1998). A lack of standardized techniques for bioindicator-based research has been implicated as a reason for high discrepancy with respect to its validity (REYERS *et al*., 2000). These criticisms can be avoided by basing indicator taxa selection on research objectives, rather than vice versa, and by following the guidelines and selection criteria offered in the literature (NEW, 1999; USEPA, 2002b). Some argue that biological indicators only provide information about that particular taxon and it is conjecture, not science, to extrapolate findings to other taxa (SIMBERLOFF, 1998). Others biologists argue that it is perfectly reasonable to infer how other taxa are faring once a relationship has been established between indicator and indicatee (MCGEOCH, 1998; CARO and OÍDOHERTY, 1999). The ramification is that this relationship must be established for each region and system and this need for empirical confirmation diminishes the practical appeal of bioindicators as management short-cuts, at least in the short-term.

The ability to infer overall biodiversity from a single index is disputed (FAITH and WALKER, 1996; SIMBERLOFF, 1998; VAN JAARSVELD *et al.,* 1998) and has been shown to be invalid in some cases (GOLDSTEIN, 1997). KREMEN (1992) found that Malagasy butterflies were inappropriate indicators of plant diversity, while PRENDERGAST *et al.* (1993) showed little correlation between the species richness of birds, butterflies, dragonflies, and aquatic plants in Britain. In addition, the presence of threatened or endangered species does not necessarily coincide with areas of high biodiversity, consequently making species at risk inadequate biodiversity indicators (BONN *et al*., 2002), and biodiversity indicators are poor indicators of rare or endemic species (REYERS *et al.,* 2000). Research in support of biodiversity indicator theory usually has a very limited realm of extrapolation, such as using one butterfly genus to indicate overall butterfly diversity (KREMEN, 1994).

The use of bioindicators in ecological studies began as early as the 1910s for use in the evaluation of water quality, particularly in streams affected by anthropogenic disturbance and pollution (KIMBERLING *et al.*, 2001). While according to PAOLETTI (1999) although not defined as such, studies began even earlier. According to TYLIANAKIS *et al.* (2004), insects are useful indicator as they represent more than half of all species and their diversity allows assessing the difference between habitats on acceptable refined scale (Table-2).

Table-2: Insect groups characteristics used as environmental indicators

Characteristics	**Description**
Richness & species diversity	Four in five species of animals are insects
Easy handling	Most species require few efforts for their capture, except toxic species. Small size of samples helps to their capture & transport.
Ecological faithfulness	Many species may have low tolerance to abiotic factors, which allows to link certain insect groups with certain habitats
Fragility to small changes	It allows selecting demographical or behavioral variables that can be measured or observed in the field, and have a close correlation with the pre selected area change.
Organism's responses	To identify levels of environmental changes

There are various approaches in bioindicator assessment. Some focus on certain taxa, but others measure the diversity of the whole community at the level of species or higher taxa (MCGEOCH, 1998; HODKINSON, 2005). Naturally, higher taxonomic resolution is more fine-scaled information on the environment. However, such a fine-scale assessment at the species level is impractical when using highly diverse taxa such as insects (Table-2). Identification of species is almost impossible for non-experts, and even for experts; the work necessitates a large amount of labor and time (BALDI, 2003; KEITH *et al*., 2004). The instant and economically reasonable approaches of using higher taxa (family or order) or functional groups for bioindicator assessment have made a presence (DEANS *et al*., 2005). A selective list of examples, where chemical changes occurring in aquatic and terrestrial ecosystems (Table-3), have been suggested as systems that could be monitored using invertebrate bioindicators. Human activities might change the normal development of these streams, especially at the fish aqua culture. Human activities, such as recreational and agricultural activities were associated with a reduction in species diversity of aquatic insect communities (WAHIZATUL *et al*., 2011).

In addition, physical and chemical disturbance, seasonal water flow, temperature, ion concentrations, food base of the stream, interaction with stream biota and substrate were also major factors in determining the composition and abundance of aquatic insects (WARD and STANFORD, 1979). Aquatic insects as a highly diverse group of insects spend all or part of their life cycle within the water in the benthic environment, are valuable indicators of environmental conditions in streams and rivers. In biomonitoring studies, insects are sensitive to many abiotic and biotic factors in the environment; though degrees of sensitivity vary. Different aquatic insect taxa have different habitat preference and pollution tolerance and absence of a sensitive taxa and presence of tolerant ones indicate water quality. Consequently, several researchers have advocated

the use of aquatic insect community structure as bioindicators of the condition of an aquatic system (ROSENBERG and RESH, 1993; SUSMITA *et al.,* 2013).

Table-3:Potential bioindicators for chemical factors in aquatic and terrestrial environments

Chemical/ pollutant	**Invertebrate group**	**References**
Aquatic		
pH/acidification	General lotic invertebrates	LARSEN *et al.,* 1996
	Lentic invertebrates	LONERGAN and RASMUSSEN, 1996
	Lentic chironomids	MOUSAVI, 2002
Nitrogen and phosphorus	Lotic insects with pathogenic microorganisms	LEMLY, 2000: LEMLY and KING 2000
	Lentic chironomids	BRODERSEN and LINDEGAARD, 1997
Heavy metals	Lentic chaoborus	CROTEAU *et al.,* 2002
	Lotic nematodes & ciliates	FENSKE and GUNTHER, 2001
	Benthic invertebrates	NELSON 2000, CAIN *et al*., 1992
	Caddisflies	AIZAWA *et al.,* 1994
Organic toxicants	Lotic nematodes & ciliates	FENSKE and GUNTHER 2001
	Cladocera	BALDWIN *et al.,* 2001
	Benthic invertebrates	GRUMIAUX *et al.,* 2000
Pesticides	Benthie invertebrates	FULTON and KEY, 2001
	Lentic zooplankton	KREUTZWEISER and FABER, 1999
	Dragonflies	TAKAMURA *et al.,* 1991
Coal mine runoff	Trichoptera	FERNANDEZ-ALAEZ *et al.,* 2002
Terrestrial		
pH/acidification	Soil microarthropods	VAN STRAALEN, 1998
Heavy/trace metals	Several soil invertebrates	CORTET *et al*., 1999; VAN STRAALEN, 1998
	Sarcophagid flies	BARTOSOVA *et al* ., 1997
Air pollution/ acid deposition	Several invertebrate	SALDIVA and BOHM 1998
	Spiders	HORVATH *et al*., 2001
	Collembola	KOPESZKI, 1997; STEINER, 1995
	Cryptostigmatic mites	STERNER, 1995
	Day flying Lepidoptera	KOZLOV *et al.,* 1996
Nitrogen input	Collembola	KOPESZKI, 1997
Pesticides	Collembola	FRAMPTON, 1997
	Bee	DORMANN *et al*., 2005

Contd… Table-3

Table-3. Contd…

Chemical/ pollutant	Invertebrate group	References
	Soil micro-arthropods	TRUBLAYEVICH & SEMENOVA, 1994
	Various soil invertebrates	CORTET *et al.,* 1999
Asbestos	Sarcophagid flies	BARTOSOVA *et al*., 1997
Crop diversity	Bees	SCHWEIGER *et al..,* 2005
	Bugs	SCHWEIGER *et al.,* 2005
	Carabid beetles	SCHWEIGER *et al.,* 2005
Composite index	Bees	HENDRICKX *et al.,* 2007

HAMILTON and SAETHER (1971) and HARDERSEN (2000) reported the potential of aquatic insects as indicators of water quality. Ephemeroptera, Plecoptera and Trichoptera are particularly sensitive to water quality. Nymphs and larvae of Ephemeroptera, Plecoptera and Trichoptera were considered integral item of the undisturbed streams (HYNES, 1960). An aquatic community considered to be good if biotic conditions will display an even distribution among these four insect families, while aquatic community with disproportionately high number of Chironomidae may indicate environmental stress (LENAT and PENROSE, 1996)). All over the world, fresh water resources have been subjected to an increasing pollution load from contaminated runoff water originated from manmade activities like domestic and industrial (BENETTI and GARRIDO, 2010), agricultural with intensive use of fertilizers and pesticides (GARCIA-CRIADO *et al*., 1999) and urbanization. These disturbances produce alteration in the chemical composition of water and in the structure of the communities of organisms living in these environments.

Aquatic indicators

Among aquatic insects, dragonflies are taken as being most sensitive to habitat disturbance (ZIA *et al.*, 2009). Their presence in any water body confirms its synthetic pollution-free status (ZIA, 2010). Ecologically they are good indicator of the condition of terrestrial as well as aquatic ecosystems (RAFI *et al*., 2009; ZIA *et al*., 2008). Butterflies and grasshoppers also have ecological fidelity and are sensitive to environmental changes and quality. According to CHEN *et al.* (2005) these insects have been successfully used as bioindicators for environmental pollution and heavy metals contaminations near industrial states and even within urban areas (NUMMELIN and HANSKI, 1989). The use of living organisms for monitoring water quality originated in Europe and now used globally (METCAFLE- SMIT, 1994). Experiences from USA and European programs have demonstrated that benthic macro-invertebrates are most useful in monitoring freshwater ecosystems (ROSENBERG and RESH, 1993).

Odonates as bioindicators

Odonates are characterized as excellent habitat indicators of present and past environmental conditions in aquatic habitats (WATSON *et al*., 1982, STEWART and SAMWAYS, 1998). They are good indicators of ecosystem health and ideal surrogate taxa for identifying freshwater biodiversity hotspots for conservation (HART *et al.* (2014). Concerning their scientific merit as appropriate bioindicator taxa, odonates satisfy most published selection criteria, rank among the top 20% for all candidate taxa, and are one of the best when considering aquatic taxa alone (CLARK and SAMWAYS, 1996). Odonates inhabit both terrestrial and aquatic habitats during their life cycle and therefore, may better reflect disturbance to the riparian buffer than other strict wetland obligates.

Regardless of their suitability, odonates have been employed as habitat indicators relatively infrequently in lotic systems (CARLE, 1979, WATSON *et al.,* 1982; CLARK and SAMWAYS, 1996; STEWART and SAMWAYS 1998), and even less frequently in lentic systems (CHOVANEC and RAAB, 1997).

Insects have strong relationship with ecology and are popularly used as bioindicators since long time (DAVIS *et al*., 2001). Globally natural ecosystems have been adversely affected by human interventions (QADIR and MALIK, 2009). Modern farming, industrialization, and increased vehicular use have led to high concentrations of heavy metals such as lead, nickel, chromium, cadmium, aluminum, mercury, and zinc in the environment (ATAFAR *et al.*, 2010). These toxic heavy metals are regularly getting into air, water, and soil, thereby becoming part of natural biogeochemical cycle (LEE *et al*., 2006). Acute and chronic effects of heavy metals on various insects are frequently reported in the form of growth inhibition, developmental abnormalities, reduced reproduction, and decreased hatchability (SILDANCHANDRA and CRANE, 2000). Insects also known as biogeochemical indicators, can be useful tool for an ecological assessment of terrestrial and aquatic ecosystems along with traditional methods of analysis (SLIVINSKY *et al.*, 2018). Predator, dragonfly (*Crocothemis servilia*) also had higher contents of heavy metals as it consumes many other insects of that area and thus accumulates high concentration of metals from aquatic environment compared to other two insect groups studied. Butterfly (*Danaus chrysippus*) being insectivorous comparatively showed less concentration of heavy metals as compared to other insect taxa studied (AZAM *et al*., 2015).

Aquatic macro-invertebrates are affected by habitat reduction and/or habitat change resulting in increased drift, lowered respiration capacity (by physically blocking gill surfaces or lowering dissolved oxygen concentrations), and changing the efficiency of certain feeding activities especially filter feeding and visual predation (LEMLY, 1982; WATERS, 1995). Metals detected in examined species are as a result of industrial effluents, agricultural runoff, vehicular smoke, domestic and sewage wastes, and use of fertilizers. As these metals are persistent and cannot be degraded by insect metabolism, hence these are accumulated at upper trophic level.

Declines in density as a response to anthropogenic disturbance and potential intrusions of fine sediment appear to be more closely associated with the order Plecoptera, (stoneflies) than with the other aquatic orders. The stoneflies, *Alloperla* and *Kathroperla perdita* (Chloroperlidae) declined in density following a clear cut (CULP and DAVIES, 1983) and *Alloperla* sp. declined after a fine sediment addition (MURPHY and HALL, 1981). Three other Plecoptera taxa also exhibited low densities in response to clearcutting: *Leuctra* (Leuctridae) (GULP and DAVIES, 1983), *Nemoura* (Nemouridae) (WEBER, 1981), or sediment addition: *Zapada* (Nemouridae) (CULP and DAVIES, 1983). CULP and DAVIES (1983) also reported declines in the Mayfly, *Cinygmula* (Heptageniidae) and reported no change in density of the Chironomidae. Densities of certain taxa such as the Chloroperlidae (stoneflies) may be important when developing a biomonitoring index. Ephemeroptera appear to be a promising order that contains sediment-tolerant and intolerant taxa from which several indicator species can be drawn. MAGNUM and WINGET (1991) found *Drunella doddsi* to be highly correlated to streams with coarse substrates. Streams with moderate to high percentages of fine sediments did not support *D. doddsi*. Ephemeroptera that were reported fine sediment intolerant or moderately intolerant both in the literature and in this research include genera *Acentrella, Caudatella, Epeorus,* and *Rithrogena* (LEMLY, 1982; MCHENRY, 1991).

There is great potential in the ability of the Fine Sediment Bioassessment Index to determine changes in aquatic organism populations and assemblages directly caused by increases in inorganic sediments. BURMAN and GUPTA (2015) noticed within 21 species of aquatic insects belonging to 14 families and 7 orders (Hemiptera, Coleoptera, Trichoptera, Ephemeroptera, Odonata, Collembola and Diptera). In premonsoon season 11 species of four orders (Hemiptera, Coleoptera, Ephemeroptera and Trichoptera) were noticed while six species of two orders (Hemiptera and Coleoptera) and seven species of three orders (Hemiptera, Coleoptera and Odonata) were recorded in monsoon and postmonsoon, respectively. In winter highest number of species i.e., 12 species of six orders (Hemiptera, Coleoptera, Ephemeroptera, Trichoptera, Collembola, and Diptera) were on record. The *Hydroptila* sp. (Trichoptera), *Bagous affinis, Dineutus* sp. (Coleoptera), *Notapictinus aurivilli* (Hemiptera) were found associated with Nitrate and low nitrate might have favoured assemblage of Trichoptera which is known as pollution sensitive group (BURMAN and GUPTA, 2015).

Family Syrphidae (Diptera) has wide distribution, and due to environmental requirements of its larvae make these flies' good bioindicators (SOMMAGGIO, 1999). Ants have been used to measure pollutant concentrations in borealis forests and Australia, and are currently used to monitor disturbed ecosystems. Bees are considered one of the most versatile and efficient bioindicators. They are used to monitor trace metals in urban environments, radioactivity after the Chernobyl disaster, pesticides and herbicides effects, industrial wastes and pollutants (URBINI *et al*., 2006). Wasps from the *Polistes* spp. and other social wasps are at the top of the food chain and, therefore, are exposed to dangerous biological concentration. As its mass larval fecal can accumulate lead up to 36 times the adult body, these wasps seem to be a promising species for pollution by lead biomonitoring (URBINI *et al*., 2006). Many insect groups have shown biomonitoring in their habitats are given in Table-4.

Terrestrial indicators

Application of bioindicators has been slow to transition from aquatic to terrestrial system. Aquatic systems possess fewer taxa than typical terrestrial systems and have fewer variable abiotic components, and the organisms have stronger associations with the physical and chemical properties of the system (MCGEOCH, 1998). In aquatic systems the presence or absence of particular organisms can be clearly related to pH, chemical contamination, temperature changes, oxygen levels and other physical and chemical characteristics of the water body (HODKINSON and JACKSON, 2005). This relationship is not as straight forward in terrestrial systems given the greater diversity, fewer quantifiable abiotic factors, and potentially more mobile organisms (MCGEOCH 1998).

Land insects are good bioindicators in various types of environmental change (Table-4). The Order Coleoptera represents approximately 20% of the total diversity of arthropods and plays roles in maintaining soil quality, population regulation of other invertebrates and energy flow, and contributes to the physical and chemistry soil formation (CARLTON and ROBISON, 1998). NUMMELIN and HANSKI (1989) and DAVIS (2000) confirmed scarabaeid beetles species have a high potential as environmental indicators in forest area or agricultural crops.

Some **lepidopteran** groups are used as environmental pollution indicators by heavy metals and carbon dioxide (CO_2 concentration) in locations close to industrial areas and even within urban areas. Presence and consequences of copper, iron, nickel, cadmium, sulfuric acid ions and other substances used in fertilizers were studied with pupae of different Geometridae and Noctuidae species (HELIÖVAARA and VÄISÄNEN, 1990), Eriocraniidae populations (KORICHEVA and HAUKIOJA, 1992), cycle duration

and newly hatched larval mortality rate from butterflies (Family: Nymphalidae), which feed on plants subjected to high CO_2 concentrations (FAJER *et al*., 1989). **Collembola** are very sensitive to changes in the soil and diversity reduction can show us pollution by heavy metals, pesticides in agricultural soils and soil water acidification by organic pollutants and waste (RUSEK, 1998). Ants are used as soil quality bioindicators and have a key role in the recovery of degraded and reforested areas (MEJER, 1984). This group, which is very sensitive to human impact, could be used as environmental indicators in different ecosystems (FOLGARAIT, 1998). Depending on the degree of the environmental change, many expert species are extinct of the site, encouraging the establishment of dominant, aggressive and generalist species, which can be used as indicators of disturbed habitats (READ, 1996).

Table-4: Bioindicator insect groups from aquatic & land environments and their role in the monitoring.

Group	**Common name**	**Biomonitoring**	**Habitat**
Order: Odonata	dragonflies and damselflies	water quality	aquatic
Order: Coleoptera Families: Gyrinidae Dytiscidae; Hydrophilidae Notonectidae; Vellidae	whirligig beetles, predaceous diving beetles, backswimmers	due to high adaptive capacity	aquatic
Order: Ephemeroptera Plecoptera	Mayflies stoneflies	due to high adaptive capacity	aquatic
Halobates	ocean-skaters	cadmium and lead	aquatic
Coleoptera: Scaribaeidae	beetles	in forest & agricultural crop	land
Coleoptera: Carabidae	beetles	biological control oil, sulfur, herbicide, CO_2, insecticide pollution	land
Family: Formicidae	ants	degraded and reforested areas recovery	land
Diptera: Sarcophagidae	flies and mosquitoes	heavy metals	land
Diptera: Syrphidae	Flies and mosquitoes	affected by diversity reduction	Land
Apis mellifera	Domestic bee	Chemical environmental Changes	land

The **ants** have shown a strong resistance to pollutants (radioactive and industrial pollutants) that may be because only about 10% of individuals fall outside the nest and exposed to the harmful pollution effects (PETAL, 1978). PECK *et al.* (1998) suggested that some ant groups have potential as biological indicators of soil conditions, crop management and assessment systems for plantations in agro-ecosystems. The impact of

ants in soil is demonstrated by leaf cutting ants in the tropics, where they are the most important agent of change in the soil, contributing to improving physical and chemical quality (CHERRET, 1989). DAVIS (2000) confirmed beetles species (Coleoptera: Scarabaeidae) having a high potential as environmental indicators in forest area or/and agricultural crops.

BARTOSOVA *et al*. (1997) showed the potential of insect species from the Family Sarcophagidae as environmental pollution indicators by heavy metals, asbestos fibers and waste chemicals. However, due to variability in the **flies'** sensitivity to insecticides and herbicides, FROUZ (1999) recommended that one must be careful when using some species of flies as chemical indicators of contaminated soil. Family Syrphidae, one of the largest families of Diptera, has wide distribution, well known taxonomy and its larvae require different environmental conditions, which makes these flies' good bioindicators. Due to environmental requirements of their larvae, these insects are particularly affected by the landscaping diversity reduction (SOMMAGGIO, 1999).

Most of the research on pollinators as bioindicators have been on population level and have focused mainly on **bees**. The pollinator strength and its population size are generally considered the most important features for plant reproduction, especially to the agricultural crops (KEVAN, 1999). Pollinators, especially honeybees (*Apis mellifera*), are considered reliable biological indicators because they show environment chemical impairment due to high mortality rate and intercept particles suspended in air or flowers. These substances can then be detected using methods of analysis (GHINI *et al*., 2004). Soil dwelling Diptera occupy five guilds: phyto-saprophages, surface scrapers, microphages, mycophages and predators and are sensitive to the impact of human activities, such as agricultural practices (type of tillage, manure, fertilizer application, pesticide application, drainage, set aside, rotation, fire, heavy metals, liming etc…). Farm situation are quite different thus there indicator need specific characteristics and suitability (Table-5).

Table-5: Insect indicators on farm land

Species indicators	**Indicator characteristics and suitability**	**References**
Epigeal Insects	Interest in terrestrial arthropods as indicators of ecological restoration since they are more sensitive (through their diversity) than vegetation	LONGCORE, 2003
Auchinorrhyncha (Hemiptera)– Planthoppers	High interest of Auchenorrhyncha as indicators of biotic conditions in grasslands.	NICKEL and HILDEBRAN DT, 2003
Coleoptera, Carabidae – (Ground beetles)	Theoretical interest in carabids as indicators but as crucial understanding of their relationship with other species is incomplete; they should be used with caution. Low interest in ground beetles as indicators of agricultural characteristics because they mainly respond to yearly climate conditions	RAINIO and NIEMELA, 2003; IRMLER, 2003

Contd… Table-5

Table-5. Contd…

Species indicators	Indicator characteristics and suitability	References
	In combination with Mollusca an appropriate indicator for biodiversity in agriculturally dominated landscapes of eastern Austria	SAUBERER *et al.,* 2004
Coleoptera, Coccinellidae (Ladybird beetles)	Interest in coccinellid life traits for bioindication of agricultural landscapes	IPERTI, 1999
Coleoptera – pollinators	Wild Coleopteran pollinators are sensitive to local and landscape floral resources, habitat fragmentation and farming practices.	RATHCKE and JULES, 1993
Coleoptera, Scarabidoidea (dung beetles)	Interest in Scarabaeine dung beetles as indicators of biodiversity, habitat transformation and pest control chemicals in agro-ecosystems. They use these dung beetles as biodiversity, ecological or environmental indicators at each of three spatial scales: regional, local, and pasture	DAVIS *et al.,* 2004
Coleoptera, Staphylinidae (Rove beetles)	Interest in Staphylinids as indicators (ecological features highlighted, but practical features seem still to be improved).	BOHAC, 1999
Hymenoptera, bees and wasps	Interest in parasitic wasps (15 families & 75 genera of Hymenoptera- Parasitica, compared with 15 species of spider (Araneae), 16 species of true bugs, 72 species of beetles, 25 families of flies (Diptera) to be used as indicators of grassland management and surrogates of grassland arthropods.	ANDERSON *et al.,* 2005
	High interest in trap-nesting bees and wasps, and their natural enemies, as indicators of habitat quality, because they represent different trophic levels and their interactions.	TSCHARNTKE *et al*., 1998
Hymenoptera, Formicidae - ants	Low interest in ants as indicators of soil function in rural environments since there is still a need for specific experiments to test the hypothesis that ants can be used as indicators of soil quality.	LOBRY DE BRUIN, 1999
Lepidoptera butterflies	Butterfly species richness and abundance were significantly increased by organic farming. Classification into different ecological groups according their general habitat requirements, requiring a structured landscape, dispersal ability, space demand, population density trophic range of caterpillars, etc. Twenty-one butterfly and four burnet species found in 1972 not observed in 2001.	RUNDLÖF *et al*., 2008; WENZEL *et al*., 2006

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Table-5. Contd…

Species indicators	**Indicator characteristics and suitability**	**References**
Lepidoptera moths	The wide range of caterpillar host plants and the ease of identification of adult moths collected by light traps provide a strong case for using moths as an indicator for farmland under different management systems.	TAYLOR, 1986; WOIWOD and STEWART, 1990; LITTLEWOOD, 2008
Orthoptera, Acrididae – grasshoppers	Pastures were the mountain grassland management type with the highest plant and grasshopper species richness ($100m^2$ plots, plots were covered by area transects).	KAMPMANN *et al*., 2008; SAUBERER *et al.*, 2008

According to ROCHA *et al.* (2018) invertebrates are more severely and quickly affected than other taxa by changes in the landscape: Insects indicator for evaluating habitats for biodiversity, condition, and structure summarized in Table-6.

Table-6: Insects indicator for evaluating habitats for biodiversity, condition, and structure

Habitat	**Invertebrate group**	**References**
Terrestrial		
General (habitat continuity)	Fungivorous beetles	SVERDRUP-THYGESON, 2001
	Diptera	FROUZ, 1999
	Coccinellid beetles	IPERTI, 1999
	Syrphid flies	HASLETT, 1997b, SOMMAGGIO, 1999
	Staphylinid beetles	BOHAC, 1999
	Rare beetles	FRANC, 1994
	Tiger beetles	PEARSON and CASSOLA, 1992
	Butterflies	BROWN and FREITAS, 2000
Landscape and habitat features	Lepidoptera, spiders, carabid beetles	JEANNERET *et al*., 2003
Agroecosystems	Heteropterous bugs	FAUVEL, 1999
	Ants	PECK *et al.,* 1998
Savanna grassland	Dung beetles	MCGEOCH *et al.,* 2002
Grassland	Collembola	GREENSLADE, 1997
Forest	Fungivorous insects	JONSELL and NORDLANDER, 2002
Boreal forest	Coleoptera	JONSSON and JONSELL, 1999
Rangeland	Ants	ANDERSEN *et al.,* 2004

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Habitat	Invertebrate group	References
Aquatic		
Aquatic ecosystems (general	Interstitial invertebrates	CLARET *et al.,* 1999
	General invertebrates	CHARVET *et al.,* 1998
River (typology)	Lotic invertebrates	CAYROU *et al.,* 2000
Stream (habitat integrity	Benthic invertebrates	BUFFAGNI and COMIN, 2000
Stream morpholo-gical integrity)	Benthic invertebrates	JANSEN *et al.,* 2000
Lake	Chironomid midges	BRODERSEN and LINDEGAARD, 1999
Ponds	Odonata & Trichoptera	BRIERS and BIGGS, 2003
Freshwater littoral	Macroinvertebrates	WHITE and IRVINE, 2003
Seasonal and temporary wetlands	Aquatic invertebrates	EULISS *et al.,* 2002
Streams	Plecoptera	HELESIC, 2001

There was a growing change of natural environments around the world, as a result of the growth of human population in recent decades. Thus, a strong understanding of insect responses to human activity is necessary both to support policy decisions for conservation and to evaluate functional consequences of human disturbance on ecosystems (NICHOLSA *et al.,* 2007). Hence, biodiversity preservation in ecosystems can provide information about maintenance of environmental resources and sustainable development. Some Lepidoptera and Diptera groups are used as heavy metal pollution indicators. In environmental pollution, bees are used to monitor trace metals in urban environments, radioactivity, and pesticide and herbicide effects. Gerrid bugs detect different iron and manganese concentrations (DA ROCHA *et al*., 2010). Since insects exhibit high fecundity, are fast breeding, easy to sample, and ethical constraints are not involved *Polistes* and other social wasps seem to be a promising species for pollution by lead biomonitoring (URBINI *et al*., 2006). *Gerris spinolae* (Lethierry & Severin) and *Brachydeutera longipes* (Hendel) appear to be suitable insect bioindicator candidates for assessing pollution particularly heavy metal in water bodies (NUMMENLIN *et al.,* 1998).

Although long-term distributional data are not available for lower latitudes and from tropical areas, movements of tropical species into more temperate areas have been reported. Five dragonflies from Cuba and the Bahamas have successfully established in Florida in 2000 (PAULSON, 2001). Species from North Africa are also moving into Spain and France. The African Monarch butterfly (*Danaus chrysippus* Linn.) established its first population in southern Spain in 1980 (HAEGER, 1999).The dragonfly, *Trithemis annulata* (Palisot de Beauvois), a widely distributed species in Africa, has expanded into the Mediterranean area colonizing the Iberian Peninsula in 1981, Corsica in 1989 and France in 1994 (BONET-BETORET, 2004). As in the case of biodiversity assessment, there are many suggested indicator taxa of habitat change, but again their comparative suitability often remains untested (Table-7).

Table-7: Insects as indicators of habitat management, degradation, restoration, and improvement

Change indicated	**Insect group**	**References**
Grassland topsoil removal	Carabid beetle	SIEREN and FISCHER, 2002
Land management practice	Ants	ANDERSEN *et al.,* 2002
	Dispersing insects	MORA *et al.,* 2004
	Dung beetles	DAVIS *et al.,* 2001
Mining disturbance in savanna	Grasshoppers	ANDERSEN *et al.,* 2001
General ecosystem health	Many invertebrates	HILTY & MERENLENDER, 2000
Landscape/ecosystem sustainability	Many invertebrates	PAOLETTI, 1999B
	Soil invertebrates	DUELLI *et al.,* 1999
Impact of genetically modified crops	Invertebrates	HAUGHTON *et al.,* 2003
Soil management	Soil invertebrates	ENAMI *et al.,* 1999
Change in general habitat quality	Bees and wasps	TSCHARNTKE *et al.,* 1998
Forest degradation	Tiger beetles	RODRIGUEZ *et al.,* 1998
	Various insects and nematodes	LAWTON *et al.,* 1998
Sheep grazing	Several insect groups	GIBSON *et al.,* 1992
Grassland managemen	Coleoptera and Orthoptera	JONAS *et al.,* 2002
Pollutant effects on forest	Scolytid beetles	GRODZKI, 1997
Forest disturbance	Butterflies	HAMER *et al*., 1997
	Moths (Arctiidae & Notodontidae)	SUMMERVILLE *et al.,* 2004
Forest management	Mycetophilid flies	OKLAND, 1994
	Forest floor invertebrates	SCHOWALTER *et al.,* 2003
	Longicorn beetles	MAETO *et al.,* 2002
Habitat fragmentation	Ants, Coleoptera, Arenaea, Diptera, other Hymenoptera	GIBB and HOCHULI, 2002
Urbanization	Carabid beetles	SUSTEK, 1992

Presence and consequences of copper, iron, nickel, cadmium, sulfuric acid ions and other substances used in fertilizers were studied with pupae of different Geometridae and Noctuidae species (HELIÖVAARA and VÄISÄNEN, 1990), Eriocraniidae populations (KORICHEVA and HAUKIOJA, 1992), cycle duration and newly hatched larval mortality rate from butterflies (Family Nymphalidae), which feed on plants subjected to high CO_2 concentrations (FAJER *et al*., 1989).

Human activities and governmental policy on forestry, farming and road planning has great effect on the abundance and distribution of butterflies, the butterfly habitats destruction thus influence global climate. Geographic range for many species has been influenced by climate. Consequently warming is expected to force species to shift their distributions by expanding into the new climatic areas and by disappearing from areas that have become climatically unsuitable (HUGHES, 2000). Numerous cases of recent

distributional shifts have been recorded for a variety of taxa from around the world (POUNDS *et al.,* 2005; PARMESAN 2006). Lepidoptera is the insect group most intensively studied. Movements of the entire species' ranges have been found in butterflies in both North America and Europe, where species shifted their ranges northward and to high elevations as a result of warming (KONVICKA *et al*., 2003; WILSON *et al*., 2005). Intensively 30 years study in UK indicated many beetles, butterflies, dragonflies, grasshoppers and aquatic bugs have moved northwards and to higher elevations during a period of warming (HICKILING *et al*., 2006). Insects have proved to be good bio-indicators of human-driven changes in the environment, such as pollution habitat loss and fragmentation (MCGEOCH, 1998). Insects have also provided examples of how biodiversity and community structure are affected by current climate change especially with range shifts in latitude and altitude (Table-8).

Table-8: Latitudinal and altitudinal range shifts reported for insect species.

Latitudinal shifts		**Altitudinal shifts**	
North expansions	South contractions	Uphill expansion	Downhill contractions
Lepidoptera (Europe)1	Lepidoptera (Europe)1	Lepidoptera (Czech Republic)9	Lepidoptera (Sierra de Guadarrama, Spain)12
Lepidoptera (UK)2	Lepidoptera (UK)2, 8	*Thaumetopoea pityocampa* (Denis & Schiffermüller (Alps, Italy)10	*Euphydryas editha*(Boisduval, 1852)(N. America)4
Euphydryas editha (Boisduval,1852) (N. America)4		Odonata (UK)7	*Parnassius appollo* (Linnaeus,1758) (Alps)13
Atalopedes campestris Boisduval, 1852 (N. America)5		Neuroptera (UK)7	
Arctia caja (Linn.), 1758)(UK)6		Coleoptera (UK)7	
Odonata (UK)7		Heteroptera (UK)7	
Neuroptera (UK)7			
Coleoptera (UK)7			
Coleoptera (UK)7			
Orthoptera (UK)7			
Sources: 1, Parmesan *et al*., 1999; 2, Hill *et al*. 2002; 3, Mikkola 1997; 4, Parmesan 1996; 5, Crozier 2003; 6, Conrad *et al*., 2002; 7, Hickling *et al.,* 2006; 8, Franco *et al*., 2006; 9, Konvicka *et al*., 2003; 10, Battisti *et al*., 2005; 11, Hódar and Zamora 2004; 12, Wilson *et al.*, 2005; 13, Descimon *et al*., 2006			

Honeybee (*Apis mellifera*) is considered reliable biological indicator because this shows environment chemical impairment due to high mortality rate and intercepts particles suspended in air or flowers. These substances can then be detected using methods of analysis (GHINI *et al*., 2004).Ants are used as soil quality bioindicators and have a key role in the recovery of degraded and reforested areas (MEJER, 1984). The ants presented a strong resistance to pollutants (radioactive and industrial pollutants)

that may be because only about 10% of individuals fall outside the nest and exposed to the harmful pollution effects (PETAL, 1978). Traditionally animal biota were used for forecasting weather conditions and for a disaster information (Table-9) as the main advantage of traditional weather forecast is its simplicity and timeliness; a person can make an independent observation without use of complicated instruments and make use of the collected information when needed without resorting to complex analysis.

There is no need for consultation with experts and in fact the indicators observed by people in their immediate environment provide more accurate information than forecasts interpolated from data of the weather stations located at distant places. Various bioindicators have been applied as useful tools to assess living conditions for organisms, traditionally in aquatic environments (ROSENBERG *et al*., 1986) and in terrestrial environments (WOODCOCK *et al.,* 2003).

Table-9: Insects used for traditional forecasting system

Butterflies: Appearance of many butterflies indicates early rainfall onset and also gives a prospect of a good season. Appearance of black butterflies in a particular area signals a very good rainfall season over that area.
Termites: Appearance of winged termites after a dry spell of some days indicates rains.
Termites: Appearance of many termites indicates near rainfall onset.
Termites: Winged termites coming out of the soil after rainfall is considered to indicate fair weather for some time.
Termites: Appearance of ants and increase in the size of anthills is also considered to indicate warming of weather and based on this observation people start to sow crops that are sensitive to low temperature.
Armyworms: Appearance of armyworms on trees during October signifies abundant rainfall in the upcoming season.
Green grasshoppers: Occurrence of more grasshoppers in a particular year indicates less rainfall and hunger.
Ants: Appearance of ants and rapidly increasing size of anthills, which are moist, indicates good rains
Ants: Appearance of ants and increase in the size of anthills indicates an increase in temperature
Ants: Ants coming out in large numbers & change in place, indicate rains
Red ants: Appearance of ants indicate imminent rainfall onset and signifies a prospect for good season. When flying ants are seen during rainy season this shows the sign of having more rainfall in the year.
Spiders: Spiders leaving their webs indicates rains
Cricket: Sound of crickets calling or chirping throughout the night indicates change in weather
Bees: When the bees come out of their hives, signs of clear weather
Bees: Sight of bees moving untimely and in large numbers towards their hives indicate bad weather and rain
Sources: RAUTELA and KARKI, 2015; ZUMA-NETSHIUKHWI *et al.,* 2013

Conclusion: Although not new, the use of bioindicators is an innovative approach for assessing various types of environmental mismanagement, including pollution, high input farming, inappropriate disposal of wastes, contamination, etc. The use of biodiversity as

a tool to assess landscape structure, transformation and fate is a valid component of policies applied to rural, managed, industrial and urbanized areas to reduce human mismanagement and alleviate pollution. India is a developing country with industrial revolution, the rapid expanding human population and its economic activities have caused a dramatic loss in biodiversity, resulting in significant disturbance to ecosystems and our living conditions. We have traditional and science based forecasting with some advantages and disadvantages. Disadvantages of traditional weather/climate forecasts/ predictions are:

i. these are only momentary but it can work well when combined with scientific forecasts/predictions;
ii. these are culture-based and interpreted differently for different areas;
iii. these do not provide predictions on the not immediate future, some seasonal indications apart;
iv. these cannot predict mid-season dry spells or their probabilities;
v. these do not indicate rainfall distributions but only when to prepare for the onset and sometimes on quality of the season to come;
vi. these are not trusted by some scientific forecast/ prediction producers as they incorrectly, perceive it as based on superstitions.

Disadvantages of science-based weather /climate forecasts/predictions are: i. these are not easily available and accessible for use in agriculture and their advantages are not documented in ways that farmers can understand; ii. These are difficult to interpret and it is not easy to make decisions based on the probabilistic information given. Today's scientists, engineers and university-educated professionals are trained to solve a narrow range of problems and have a limited ability to deal with complex systems.

Thus applications of bioindicators can be expected to help not only in improving the environment, but also in augmenting awareness of the living creatures around so that a better appreciation of the crucial role in sustaining life on the planet is obtained. Many of us consider insects as pests that must be disinfested; biologists and entomologists have been trained to focus more on pest problems, rather than their potential usefulness. According to CHANDRA (2011) the insect diversity of Sikkim (5941 species) is the greatest, followed by those of West Bengal (5818 species), Meghalaya (5118 species) and Uttarakhand (4160 species). All these states are quite well explored, but many more species are expected, possibly up to 15,000 species. The states of Chhattisgarh, Delhi, Gujarat, Jammu and Kashmir, Haryana, Jharkhand, Mizoram, and Nagaland are the least explored, and fewer than 1000 species have been reported from each of them, and the number of species may be up to 4000 species. There is a need to increase knowledge of these undervalued insects in order to better appreciate the many benefits including their role as bioindicator that humans derive from their existence.

REFERENCES

ANDERSEN, A. N., HOFFMANN, B. D., MULLER, W. J. and GRIFFITHS, A. D. 2002. Using ants as bioindicators in land management: simplifying assessment of ant community responses. *Journal of Applied Ecology* **39**: 8–17.

ANDERSEN, A. N., LUDWIG, J. A., LOWE, L. M. and RENTZ, D. C. F. 2001. Grasshopper biodiversity and bioindicators in Australian tropical savannas: responses to disturbance in Kakadu National Park. *Austral Ecology* **26**: 213–222.

ANDERSEN, E., ELBERSEN, B., GODESCHALK, F. and VERHOOG, D. 2007 Farm management indicators and farm typologies as a basis for assessments in a changing policy environment. *Journal of Environmental Management*, **82**(3): 353-362.

ATAFAR, Z. A., MESDAGHINIA, J., NOURI HOMAEE, M., YUNESIAN, M., AHMADIMOGHADDAM, M. and MAHVI, H. 2010 “Effect of fertilizer application on soil heavy metal concentration,” *Environmental Monitoring and Assessment*, **160**: 1 - 4.

AZAM, I., AFSHEEN, S., ZIA, A., JAVED, M., SAEED, R., SARWAR, M. K. and MUNIR, B. 2015. Evaluating Insects as Bioindicators of Heavy Metal Contamination and Accumulation near Industrial Area of Gujrat, Pakistan *BioMed Research International Volume. Article ID 942751, 11 pages http://dx.doi.org/10.1155/2015/942751*.

BALDI, A. 2003. Using higher taxa as surrogates of species richness: a study based on 3700 Coleoptera, Diptera, and Acari species in Central-Hungarian reserves*. Basic and Applied Ecology* **4**: 589-593.

BALDWIN, W. S., BAILEY, R., LONG, K. E. and KLAINE, S. 2001. Incomplete ecdysis is an indicator of ecdysteroid exposure in *Daphnia magna*. *Environ.Toxico.& Chem.,* **20**: 1564–1569.

BARMAN, B. and GUPTA, S. 2015. Aquatic insects as bio-indicator of water qualityA study on Bakuamari stream, Chakras hila Wildlife Sanctuary, Assam, North East India. *Journal of Entomology and Zoology Studie*s, **3**(3): 178-186.

BARTOSOVA, M., GLOVINOVA, E. and POVOLNY, D. 1997. Use of flesh-flies (Diptera, Sarcophagidae) for ecotoxicological bioindication. *Ekol.-Bratislava*, **16**: 319- 322.

BATTISTI, A., STASTNY, M., NETHERER, S., ROBINET, C., SCHOPF, A., ROQUES, A. and LARSSON, S. 2005. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecological Appli- Applic.* **15**: 2084–2096.

BENETTI, C. J. and GARRIDO, J. 2010. The influence of stream habitat and water quality on water beetles assemblages in two rivers in northwest Spain. *Vie et milieu*, **60**(1): 53-63.

BOHAC, J. 1999. Staphylinid beetles as bioindicators. *Agric. Ecosyst. & Environ.* **74**: 357–372.

BONET-BETORET, C. 2004. Expansión de *Trithemis annulate* en Europa en los años 80 y 90*. Boletín de la Sociedad Entomológica Aragonesa* **27**: 85–86.

BONN, A., RODRIGUES, A. S. L. and GASTON, K. J. 2002. Threatened and endemic species: are they good indicators of patterns of biodiversity on a national scale? *Ecology Letters* **5**: 733-741.

BRIERS, R. A. and BIGGS, J. 2003. Indicator taxa for the conservation of pond invertebrate diversity. *Aquatic Conservation-Marine and Freshwater Ecosystems* **13**: 323–330.

BRODERSEN, K. P. and LINDEGAARD, C. 1997. Significance of subfossil chironomid remains in classification of shallow lakes. *Hydrobiologia* **342**: 125–132.

BROWN, K. S., and FREITAS, A. V. L. 2000. Atlantic forest butterflies: Indicators for landscape conservation*. Biotropica* **32**: 934–956.

CAIRNS, J. and PRATT, J. R. 1993. A history of biological monitoring using benthic macroinvertebrates, *In: Rosenberg D.M. and Resh V.H. (eds.), Freshwater Biomonitoring and Benthic Macroinvertebrates Chapman and Hall New York,* 10-27

CANNON, R. J. C. 1998. The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Global Change Biol*. **4** : 756-796.

CARCHINI, G. and ROTA, E. 1985. Chemico-physical data on the habits of rheophile Odonata from central Italy*. Odonatologica* **14**: 239-245.

CARLE, F. L. 1979. Environmental monitoring potential of the Odonata, with a list of rare and endangered Anisoptera of Virginia, USA. *Odonatologica* **8**: 319-323.

CARO, T. M. and O'DOHERTY, G. 1999. On the use of surrogate species in conservation biology. *Conservation Biology* **13**: 805-814.

CARROLL, S. S. and PEARSON, D. L. 1998. Spatial modeling of butterfly species richness using tiger beetles (Cicindelidae) as a bioindicator taxon. *Ecological Applic.* **8**: 531-543

CAYROU, J., COMPIN, A., GIANI, N. and CEREGHINO, R. 2000. Species associations in lotic macroinvertebrates and theiruse for river typology. Example of the Adour-Garonne drainage basin (France). *Journal of Limnology* **36**:189–202.

CHANDRA, KAILASH, 2011. Insect Fauna of States and Union Territories in India, *In Arthropods and their Conservation in India (Insects & Spiders) ed. By V.P. Uniyal and Aseem Shrivastava,* pp 189-218.

CHEN, T. B., ZHENG, Y. M., LEI, M., HUANG, ZE-CHUN; WU, HONG-TAO; CHEN, HUANG; FAN, KE-KE; YU, KE; WU, XIAO and TIAN, QIN-ZHENG, 2005. Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China, *Chemosphere*, **60**: 542–551.

CHOANEC, A. and RAAB, R. 1997. Dragonflies (Insects, Odonata) and the ecological status of newly created wetlands: Examples for long-term bioindication programmes*. Limnologica* **27**(3-4): 381-392.

CHRISTENSEN, J. H., HEWITSON, B., BUSUIOC ,A., CHEN, A., GAO, X., HELD, I., JONES, R., KOLLI, R. K., KWON, W. T., LAPRISE, R., MAGAÑA RUEDA, V., MEARNS, L., MENÉNDEZ, C. G., RÄISÄNEN, J., RINKE, A., SARR, A. and WHETTON, P. 2007. Regional Climate Projections. In: Climate Change 2007: The Physical Science Basis. *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M and Miller H L (eds)]. Cambridge University Press. Cambridge, United Kingdom and New York, NY, USA*; 2007.

CLARET, C., MARMONIER, P., DOLE-OLIVIER, M. J., DES CHATELLIERS, M. C., BOULTON, A. J. and CASTELLA, E. 1999. A functional classification of interstitial invertebrates: supplementing measures of biodiversity using species traits and habitat affinities. *Archiv fu¨r Hydrobiologie* **145**: 385–403.

CLARK, T. E. and SAMWAYS, M. J. 1996. Dragonflies (Odonata) as indicators of biotype quality in the Kruger national Park, South Africa*. J. Applied Ecology* **33**: 1001-1012.

CONRAD, K. F., WOIWOOD. I. P. and PERRY, J. N. 2002. Long-term decline in abundance and distribution of the garden tiger moth (*Arctia caja*) in Great Britain. *Biological Conservation* **106**: 329–337.

CORTET, J., GOMOT-DE VAUFLERY, A., POINSOT-BALAGUER, N., GOMOT, L., TEXIER, C. and CLUZEAU, D. 1999. The use of invertebrate soil fauna in monitoring pollutant effects.European, *Journal of Soil Biology* **35**:115–134.

CROTEAU, M. N., HARE, L., CAMPBELL, P. G. C. and COUILLARD, Y. 2002. Metallothionein-like metal-binding protein in the biomonitor *Chaoborus*: occurrence and relationship to ambient metal concentrations in lakes. *Environmental Toxicology and Chemistry* **21**:737–741.

CROZIER, L. 2003. Winter warming facilitates range expansion: cold tolerance of the butterfly, *Atalopedes campestris*. *Oecologia* **135**: 648–656.

CULP, J. M. and DAVIES, R. W. 1983. An assessment of the effects of streambank clear-cutting, on macroinvertebrate communities in a managed watershed. *Canadian Technical Report for Fisheries and Aquatic Sciences, Ottawa, ON, Canada,* # 1208.

DALLINGER, R. 1994. Invertebrate organisms as biologicalindicators of heavy-metal pollution. *Applied Biochemistry and Biotechnology* **48**: 27–31.

DAVIS, A. 2000. Does reduced-impact logging help preserve biodiversity in tropical rainforest? A case study from Borneo using dung beetles (Scarabaeoidea) as indicators. *Environ. Entomol*. **29**(3): 469-473.

DAVIS, A. J., HOLLOWAY, J. D., HUIJBREGTS, H., KRIKKEN, J., HKIRK-SPRIGGS, A. and SUTTON, S. L. 2001. Dung beetles as indicators of change in the forests of northern Borneo. *Journalof Applied Ecology* **38**: 593–616.

DAVIS, A. L., SCHOLTZ, C. H., DOOLEY, P., BHAM, N., and KRYGER, U. 2004. Scarabaeine dung beetles as indicators of biodiversity, habitat transformation and pest control chemicals in agro-ecosystems. *South African J. Science* **100**: 415–424.

DE BRUYN, L. A. L.1999. Ants as bioindicators of soil function in rural environments. *Agr. Ecosyst. Environ*. **74**: 425–441.

DESCIMON, H., BACHELARD, P., BOITIER, E. and PIERRAT, V. 2005. Decline and extinction of *Parnassius apollo* populations in France – continued. – *In: E. Kühn, R. Feldmann, J. Thomas & J. Settele (Eds.), Studies on the Ecology and Conservation of Butterflies in Europe: 114–115.*

DUELLI, P., OBRIST, M. K. and SCHMATZ, D. R. 1999. Biodiversity evaluation in agricultural landscapes: above-ground insects. *Agriculture, Ecosystems and Environment* **74**: 33–64.

EKSCHMITT, K., STIERHOF, T., DAUBER, J., KREIMES, K. and WOLTERS, V. 2003 On the quality of soil biodiversity indicators: abiotic and biotic parameters as predictors of soil faunal richness at different spatial scales. *Agric.Ecosystems & Environ.* **98**: 273-283.

ENAMI, Y., SHIRAISHI, H. and NAKAMURA, Y. 1999. Use of soil animals as bioindicators of various kinds of soil management in northern Japan. *Jarq—Japan Agricultural Research Quarterly,* **33**: 85-89.

EULISS, N. H., MUSHET, D. M. and JOHNSON, D. H. 2002. Using aquatic invertebrates to delineate seasonal and temporarywetlands in the prairie pothole region of North America. *Wetlands* **22**: 256–262.

FAITH, D. P. and WALKER, P. A. 1996. How do indicator groups provide information about the relative biodiversity of different sets of areas?- on hotspots, complementarily and pattern-based approaches. *Biodiversity Letters* **3**: 18-25.

FAJER, E. D., BOWERS M. D. and BAZZAZ, F. A. 1989 The effects of enriched carbon dioxide atmospheres on plant-insect herbivore interactions. *Science*, **243**: 1198–1199.

FAUVEL, G. 1999. Diversity of Heteroptera in agroecosystems: role of sustainability and bioindication. *Agriculture Ecosystems and Environment* **74**: 275–303.

FERNANDEZ-ALAEZ, C., SOTO, J., FERNANDEZ-ALAEZ, M. and GARCIA-CRIADO, F. 2002. Spatial structure of the caddisfly (Insecta, Trichoptera) communities in a river basin in NW Spain affected by coal mining. *Hydrobiologia* **487**: 193–205.

FRAMPTON, G. K. 1997. The potential of Collembola as indicators of pesticide usage: evidence and methods from the UK arable ecosystem. *Pedobiologia* **41**: 179–184.

FRANC, V. 1994. On the occurrence and bioindicative value of several rare species of the family Tetratomidae andMelandryidae (Coleoptera) in Slovakia. *Biologia* **49**: 723– 728.

FROUZ, J. 1999. Use of soil dwelling Díptera (Insecta, Díptera) as bioindicaors: a review of ecological requirements & response to disturbance. *Agr. Ecosyst. Environ.,* **74**: 167–186.

FULTON, M. H. and KEY, P. B. 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. *Environmental Toxicology and Chemistry* **20**: 37–45.

GARCIA-CRIADO, F., TOME, A., VEGA, F. J. and ANTOLIN, C. 1999. Performance of some diversity and biotic indices in rivers affected by coal mining in northwestern Spain. *Hydrobiologia* **394**: 209–217.

GASTON, K. J. 2000. Biodiversity: higher taxon richness. *Progress Physical Geog.,* **24**: 117-127.

GHINI, S., FERNÁNDEZ, M., PICÓ, Y., MARÍN, R., FINI, F., MAÑES, J. and GIROTTI, S. 2004. Occurrence and distribution of pesticides in the province of Bologna, Italy, using honeybees as bioindicators*. Arch. Environ. Contam.Toxicol.,* **47**: 479–488.

GIBB, H., and HOCHULI, D. F. 2002. Habitat fragmentation inan urban environment: large and small fragments support arthropod assemblages. *Biological Conserv.***106**: 91–100.

GIBSON, C. W. D., BROWN, V. K., LOSITO, L. and MCGAVIN, G. C.1992. The response of invertebrate assemblies to grazing*. Ecography* **15**: 166–176.

GOLDSTEIN, P. Z. 1997. How many things are there? A reply to Oliver and Beattie, Beattie and Oliver, Oliver and Beattie, and Oliver and Beattie. *Conserv. Biology* **11**(2): 571-574.

GREENSLADE, P. 1997. Are Collembola useful as indicators of the conservation value of native grasslands? *Pedobiologia* **41**: 215–220.

GRODZKI, W. 1997. *Pityogenes chalcographus* (Coleoptera, Scolytidae)—An indicator of man-made changes in Norwayspruce stands. *Biologia* **52**: 217–220.

HAEGER, J. F. 1999. *Danaus chrysippus* (Linnaeus 1758) en la Península Iberica: migraciones o dinámica de metapoblaciones? – *Shilap* **27**: 423–430.

HAMER, K. C., HILL, J. K., LACE, L. A. and LANGAN, A. M. 1997. Ecological and biogeographical effects of forest disturbance on tropical butterflies of Sumba, Indonesia. *Journal of Biogeography* **24**: 67–75.

HAMILTON, A. L. and SAETHER, O. A. 1971. The occurrence of characteristic deformities in the chiromomid larvae of several Canadian lakes. *The Canad. Entom*., **103**: 363 – 68.

HARDERSEN, S. 2000. The role of behavioural ecology of damselflies in the use of fluctuating asymmetry as a bioindicator of water population. *Ecolog. Entomol*., **25**: 45 – 53.

HART, L. A., BOWKER, M. B., TARBOTON, W. and DOWNS, C. T. 2014. Species composition, distribution and habitat types of Odonata in their Simangaliso Wetland Park, KwaZulu-Natal, South Africa and the associated conservation implications. *PLoS ONE 9(3): e92588 (doi:10.1371/journal.pone.0092588).*

HELIÖVAARA, K. and VÄISÄNEN, R. 1990 Heavy-metal contents in pupae of *Bupalus piniarus* (Lepidoptera: Geometridae) and *Panolis flammea* (Lepidoptera: Noctuidae) near an industrial source. *Environ. Entomol*., **19**: 481 – 485.

HELLAWELL, J. M. 1986. Biological indicators of freshwater pollution and environmental management. *In, Pollution Monitoring Series, K. Mellanby (ed). Elsevier Applied Science Publishers, London, UK*, pp 546.

HENDRICKX, F., VAN WINGGERDEN, W., MAELFAIT, JEAN- PIERRE and BUGTER, R. J. F. 2007. How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. *J. Appl. Ecology,* **44**(2): 340-351.

HICKLING, R., ROY, D. B., HILL, J. K., FOX, R. and THOMAS, C. D. 2006. The distributions of a wide range of taxonomic groups are expanding pole wards. – *Global Change Biology* **12**: 450–455.

HILL, J. K., THOMAS, C. D., FOX, R., TELFER, M. G., WILLIS, S. G., ASHER, J. and HUNTLEY, B. 2002. Responses of butterflies to twentieth century climate warming: implications for future ranges. – *Proc. Royal Society of London* B **269**: 2163–2171.

HILTY, J. and MERENLENDER, A. 2000. Faunal indicator taxa selection for monitoring ecosystem health. *Biological Conservation* **92**: 185-197.

HÓDAR, J. A. and ZAMORA, R. 2004. Herbivory and climatic and climatic warming: a Mediterranean out-breaking caterpillar attacks a relict boreal pine species. – *Biodiversity and Conservation* **13**: 493–500.

HODKINSON, I. D. 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. *Environmental Management* **35**: 649-666.

HORVATH, R., MAGURA, T. and SZINETAR, C. 2001. Effects of emission load on spiders living on black pine. *Biodiversity and Conservation* **10**: 1531–1542.

HUGHES, L. 2000. Biological consequences of global warming: Is the signal already apparent? – *Trends in Ecology and Evolution* **15**: 56–61.

HYNES, H. B. N. 1960. The biology of polluted waters. *Liverpool Univ. Press, UK,* pp 202.

IPERTI, G. 1999. Biodiversity of predaceous coccinellidae in relation to bioindication and economic importance. Agriculture *Ecosystems and Environment*, **74**: 323-342.

IRMLER, U. 2003. The Spatial and Temporal Pattern of Carabid beetles on Arable Fields in Northern Germany (Schleswig-Holstein) and Their Value as Ecological Indicators, *Agriculture, Ecosystem and Environment,* **98**(1-3): 141-151.

IUCN. 1989. From Strategy to Action: The IUCN Response to the Report of the World Commission on Environment and Development. *IUCN, Gland, and Cambridge, UK.*

JANSEN, A. 1997. Terrestrial invertebrate community structureas an indicator of the success of a tropical rainforest restoration project. *Restoration Ecology* **5**: 115–124.

JANSEN, W., OHMER, J., KAPPUS, B., BEITER, T., BREITINGER, B. and Hock, C. 2000. Benthic invertebrate and fish communitiesas indicators of morphological integrity in the Enz River. *Hydrobiologia* **422**: 331–342.

JEANNERET, P., SCHUPBACH, B.; PFIFFNER, L. and WALTER, T. 2003.Arthropod reaction to landscape and habitat features in agricultural landscapes. *Landscape Ecology* **18**: 253–263.

JONAS, J. L., WHILES, M. R. and CHARLTON, R. E. 2002. Aboveground invertebrate responses to land management differences in a Central Kansas grassland. *Environmental Entomology* **31**: 1142–1152.

JONSSON, B. G., and JONSELL, M. 1999. Exploring potential biodiversity indicators in boreal forests. *Biodiversity and Conservation* **8**: 1417–1433.

KEITH, S., SUMMERVILLE, L. M. R. and THOMAS O. C. 2004. Forest moth taxa as indicators of lepidopteran richness and habitat disturbance: a preliminary assessment. *Biological Conservation* **116**: 9-18.

KIMBERLING, D. N., KARR, J. R. and FORE, L. S. 2001. Measuring human disturbance using terrestrial invertebrates in the shrub-steppe of eastern Washington (USA*). Ecological Indicators* **1**: 63-81.

KOPESZKI, H. 1997. An active bioindication method for thediagnosis of soil properties using Collembola. *Pedobiologia* **41**:159–166.

KORICHEVA, J. and HAUKIOJA, E. 1992. Effects of air pollution on host plant quality, individual performance, and population density of *Eriocrania miner* (Lepidoptera: Eriocraniidae). *Environ. Entomol*., **26**(6): 1386 – 1392.

KREMEN, C. 1994. Biological inventory using target taxa: a case study of the butterflies of Madagacar. *Ecological Applications* **4**: 407-422.

KREUTZWEISER, D. P. and FABER, M. J. 1999. Ordination of zooplankton community data to detect pesticide effects in pond enclosures. *Archives of Environmental Contamination and Toxicology* **36**: 392–398.

LANDRES, P. B., VERNER, J. and THOMAS, J. W. 1988. Ecological uses of vertebrate indicator species: a critique. *Conservation Biology* **2**: 316-328.

LARSEN, J., BIRKS, H. J. B., RADDUM, G. and FJELLHEIM, A. 1996. Quantitative relationships of invertebrates to pH in Norwegian rivers*. Hydrobiologia* **328**: 57–74.

LAWTON, J. H., BIGNELL, D. E., BOLTON, B., BLOEMERS, J. F., EGGLETON, P., HAMMOND, P. M., HODDA, M., HOLT, R. D., LARSEN, T. B., STORK, N. E., SRIVASTAVA, D. S. and WATT, A. D. 1998. Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. *Nature* **391**: 72–76.

LEE, S. L., LI, X., SHI W., CHEUNG, S. C. N. and THORNTON, I. 2006. Metal contamination in urban, suburban, and country park soils of Hong Kong: a study based on GIS and multivariate statistics, *Science of the Total Environment,* **356**(1–3): 45–61.

LEMLY, A. D. 1982. Modification of benthic insect communities in polluted streams: combined effects of sedimentation and nutrient enrichment. *Hydrobiologia* **87**: 229-245.

LEMLY, A. D. 2000. Using bacterial growth on insects to assess nutrient impacts in streams. *Environmental Monitoring and Assessment* **63**: 431–446.

LEMLY, A. D. and KING, R. S. 2000. An insect-bacteria bioindicator for assessing detrimental nutrient enrichment in wetlands. *Wetlands* **20**: 91–100.

LENAT, D. R. and PENROSE, D. L. 1996. History of the EPT taxa richness metric*. Bull. North Am. Benthol Soc.* **13**: 305–306.

LITTLEWOOD, N. A. 2008 Grazing impacts on moth diversity and abundance on a Scottish upland estate. Insect Conservation and Diversity, **1**(3): 151-160.

LOBRY DE BRYUYN L.A. 1999. Ants as bioindicators of soil function in rural environments. *Agr. Ecosyst. Environ.* **74**: 425–441.

LONERGAN, S. P. and RASMUSSEN, J. B. 1996. A multi-taxonomic indicator of acidification: isolating the effects of pH from other water-chemistry variables. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 1778–1787

LONGCORE, T. 2003. Terrestrial Arthropods as Indicators of Ecological Restoration Success in Coastal Sage Scrub (California, U.S.A.). *Restoration Ecology*, **11**(4): 397-409.

MAETO, K., SATO, D. and MIYATA, 2002. Species diversity of longicorn beetles in humid warm-temperate forests: the eimpact of forest management practices on old-growth forest species in southwestern Japan. *Biodiversity and Conservation* **11**: 1919–1937.

MANGUM, F.A. and WINGET, R. N. 1991. Environmental profile of *Drunella* (*Eatonella) doddsi* (Needham) (Ephemeroptera: Ephemerellidae). *J. Freshwater Ecology* **6**: 11-22.

MCGEOCH, M. A. 1998. The selection, testing and application of terrestrial insects as bioindicators*. Biological Review,* **73**: 181-201.

MCGEOCH, M. A. and CHOWN, S. L. 1998. Scaling up the value of bioindicators. trends in ecology and evolution **13**: 46-47.

MEJER, J. D. 1984. The influence of ants on seeding operations in Northern Australian mined areas. *Reclam. Reveg. Res.* **2**: 299 – 313.

METCALFE-SMITH, J. L. 1994. Biological water-quality assessment of rivers: use of macroinvertebrate communities. *In: Calow P, Petts GE, Editors. The Rivers Handbook: Blackwell Scientific Publications, London*, **2**: 144- 170.

MIKKOLA, K., 1997. Population trends of Finnish Lepidoptera during 1961–1996. *Entomologica Fennica* **3**: 121–143.

MORA, F., ALBIGNAC, R., J. C. ROBERT, J. C., CRETIN, J. Y., PROUTEAU, C. and ROBERT. J. Y. 2004. The spatiotemporal structure of the mobile insect communities as a bioindicator in forest and grassland management. *Revue d'Ecologie-La Terre et la Vie* **59**: 229–241.

MOUSAVI, S. K. 2002. Boreal chironomid communities and their relations to environmental factors- the impact of lake depth, size and acidity. *Boreal Environment Res.* **7**: 63–75.

MURPHY, M. L. and HALL, J. D. 1981. Varied effects of clear-cut logging on predators and their habitat in small streams of the Cascade Mountains, Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* **38**: 137-145.

NEW, T. R. 1999. Entomology and nature conservation*. European J. Entomology* **96**(1): 11-17.

NICHOLSA, E., LARSENB, T., SPECTORA, S., DAVISE, A. L., ESCOBARC, F., FAVILAD, M. and VULINECE*,* K. 2007. Global dung beetle response to tropical forest modification and fragmentation: A quantitative literature review and meta-analysis. *Biological Conserv*. **137**: 1–19.

NICKEL, H. and HILDEBRANDT, J. C. 2003. Auchenorrhyncha communities as indicators of disturbance in grasslands (Insecta, Hemiptera)- a case study from the Elbe flood plains*. Agriculture Ecosystems & Environment* **98**: 183–199.

NOSS, R. F. 1990. Indicators for monitoring biodiversity: a hierarchical approach*. Conservation Biology* **4**: 355-364.

NUMMELIN, M. and HANSKI, I. 1989. Dung beetles of the Kibale Forest, Uganda; Comparison between virgin and managed forest, *J. Trop. Ecol.*, **5**: 349 – 352.

NUMMENLIN, M.; LODINEUS, M. and TULISALO, E. 1998. Water strider as bioindicators of heavy metals*, Entomologica Fennica*, **8**: 185–191.

OKLAND, B. 1994. Mycetophilidae (Diptera), an insect group vulnerable to forestry practices-a comparison of clear-cut, managed and semi-natural spruce forests in southern Norway*. Biodiversity and Conservation* **3**: 68–85.

OLIVER, I. and BEATTIE, A. J. 1993. A possible method for the rapid assessment of biodiversity. *Conservation Biology* **7**(3): 562-568.

PAOLETTI, M. G. 1999. Using bioindicators based on biodiversity to assess landscape sustainability. *Agriculture Ecosystems and Environment* **74**: 1–18.

PARMESAN, C.; RYRHOLM, N.; STEFANESCU, C.; HILL, J. K.; THOMAS, C. D.; DECIMON, H.; HUNTLEY, B.; KAILA, L.; KULLBERG, J.; THOMAS, J. A. and WARREN, M. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming*. Nature* **399**: 579–583.

PARMESAN, C., 1996. Climate and species' range. *Nature* **382**: 765–766.

PAULSON, D. R. 2001. Recent Odonata records from southern Florida: effects of global warming? *International Journal of Odonatology* **4**: 57–69.

PEARSON, D. L. and CASSOLA, F. 1992. Worldwide species richness patterns of tiger beetles (Coleoptera, Cicindelidae): indicator taxon for biodiversity and conservation studies. *Conservation Biology* **6**: 376–391.

PECK, S. L., MCQUAID, B and CAMPBELL, C. L. 1998. Using ant species as a biological indicator of agro-ecosystem condition. *Environmental Entomology* **27**: 1102–1110.

PETAL, J. 1978. Adaptation of ants to Industrial Pollution. *Memorabilia Zool*., **29**: 99

POUNDS, J. A., FOGDEN, M. P. L. and MASTERS, K. L. 2005. Responses of natural communities to climate change in a highland tropical forest. Case study – *In: T. Lovejoy & L. Hannah (Eds.), Climate Change &Biodiv., Yale Univ. Press, New Haven,* pp 70-74.

PRENDERGAST, J. R. and EVERSHAM, B. C. 1997. Species richness covariance in higher taxa: Empirical tests of the biodiversity indicator concept*. Ecography* **20**(2): 210-216.

QADIR, A. and MALIK, R. N. 2009. Assessment of an index of biological integrity (IBI) to quantify the quality of two tributaries of river Chenab, Sialkot, Pakistan, *Hydrobiologia*, **621**(1): 127–153.

RAFI, M. A., KHAN, M. R., ZIA, A. and SHEHZAD, A. 2009. Diversity of Odonata in district Poonch and Sudhnoti of Kashmir Valley-Pakistan, with a new record for the country, *Halteres*, **1**(1): 28–35.

RAUTELA, P. and KARKI, B. 2015, Weather forecasting: Traditional knowledge of the people of Uttarakhand Himalaya. *JGEESI*, **3**(3): 1-14.

REYERS, B.; VAN JAARSVELD, A. S. and KRUGER, M. 2000. Complementarity as a biodiversity indicator strategy. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **267**(1442): 505-513.

RODRIGUEZ, J. P., PEARSON, D. L. and BARRERA, R. 1998. A testfor the adequacy of bioindicator taxa: are tiger beetles(Coleoptera: Cicindelidae) appropriate indicators for monitoring the degradation of tropical forests in Venezuela? *Biol. Conserv.***83**: 69–76.

ROSENBERG, D. M. and RESH, V. H.1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: Rosenberg, D. M., Resh, V. H., editors. Freshwater biomonitoring and benthic macroinvertebrates: *Chapman and Hall, New York,* 1-9.

ROSENBURG, D.M. and RESH, V. H.. 1996. Use of aquatic insects in biomonitoring. *In, An introduction to the aquatic insects of North America (3rd ed.). R.W. Merritt and K.W. Cummins (eds). Kendall/ Hunt Publishing Company, Dubuque, IA*. 862pp.

RUNDLÖF, M., SMITH, H. G. and BENGTSSON, J. 2008. Local and landscape effects of organic farming on butterfly species richness and abundance. *Journal of Applied Ecology*, **45:** 813-820.

RUSEK, J. 1998. Biodiversity of Collembola and their functional role in the ecosystem. *Biodivers. Conserv.,* **7**: 1207 – 1219.

SALDIVA, P. H. N., and BOHM, G. M. 1998. Animal indicators ofadverse effects associated with air pollution*. Ecosystem Health* **4**: 230–235.

SAMWAYS, M. J. and STETLER, N. S. 1996. Dragonfly (Odonata) distribution patterns in urban and forested landscapes, and recommendations for riparian management. *Biological Conservation* **78**(3): 279-288.

SAUBERER, N., ZULKA, K. P., ABENSPERG-TRAUN, M., BERG, H.M., BIERINGER, G., MILASOWSZKY, N., MOSER, D., PLUTZAR, C., POLLHEIMER, M., STORCH, C., TROSTL, R., ZECHMEISTER, H. G. and GRABHERR, G. 2004. Surrogate taxa for biodiversity in agricultural landscapes of eastern Austria. *Biol. Conserv.* **117**: 181–190.

SCHOWALTER, T. D., ZHANG, Y. L. and RYKKEN, J. 2003. Litter invertebrate responses to variable density thinning in western Washington forest. *Ecol. Appls.* **13**: 1204-1211.

SCHWEIGER, O., MAELFAIT, J. P., VAN WINGERDEN, W., HENDRICKX, F., BILLETER, R., SPEELMANS, M., AUGENSTEIN, I., AUKEMA, B., AVIRON, S., BAILEY, D., BUKACEK, R., BUREL, F., DIEKÖTTER, T., DIRKSEN, J., FRENZEL, M.., HERZOG, F., LIIRA, J., ROUBALOVA, M. and BUGTER, R.. 2005 Quantifying the impact of environmental factors on arthropod communities in agricultural landscapes across organizational levels and spatial scales. *J. Applied Ecology*, **42**(6): 1129-1139.

SILDANCHANDRA, W. and CRANE, M. 2000. Influence of sexual dimorphism in Chironomus riparius Meigen on toxic effects of cadmium, *Environ. Toxic. & Chem.***19**(9): 2309–2313.

SIMBERLOFF, D. 1988. Flagships, umbrellas, and keystones: is single-species management passe in the landscape era? *Biological Conservation* **83**: 247-257.

SLIVINSKY, G., TEMRESHEV, I., ISSENOVA, G., KOZHABAEVA, G. and SAGITOV, A. 2018. Insects as bioindicators of industrial pollution in Kazakhstan. *Bioscience Research*, **15**(2): 1270-1282.

SOMMAGGIO, D. 1999. Syrphidae: can they be used as environmental bioindicators? *Agr. Ecosyst. Environ.,* **74**: 343– 356.

STEINER, W. A. 1995. Influence of air-pollution on mossdwelling animals. 3. Terrestrial fauna, with emphasis onOribatida and Collembola. *Acarologia* **36**:149–173.

STEWART, D. A. B. and SAMWAYS, M. J. 1998. Conserving dragonfly (Odoanta) assemblages relative to river dynamics in an African savanna game reserve. *Conservation Biology* **12**(3): 683-692.

SUMMERVILLE, K. S., RITTER, L. M. and CRIST, T. O. 2004. Forest moth taxa as indicators of lepidopteran richness and habitat disturbance: a preliminary assessment. *Biological Conservation* **116**: 9–18.

SUSMITA, G., SUSHMITA, D. and PINKI, P. 2013. Use of aquatic insects in water quality assessment of ponds around two cement factories of Assam, India. *Int. Res. J. Environ. Sci*. **2** (7): 15-19.

SUSTEK, Z. 1992. Changes in the representation of carabid lifeforms along an urbanization gradient (Coleoptera, Carabidae). *Biologia* **47**: 417–430.

SVERDRUP-THYGESON, A. 2001. Can continuity indicator species predict species richness or red-listed species ofsaproxylic beetles? *Biodiversity and Conservation* **10**: 815– 832.

TAKAMURA, K.; HATAKEYAMA, S. and SHIRAISHI, H. 1991. Odonate larvae as an indicator of pesticide contamination*. Applied Entomology and Zoology* **26**(3): 321-326.

TAYLOR, L. R. 1986. Synoptic dynamics, migration and the Rothamsted Insect Survey. *Journal of Animal Ecology,* **55:** 1-38.

TRUBLAYEVICH, Z. N., and SEMENOVA, Y. N. 1994. The population of small arthropods as an indicator of soil pollution.*Eurasian Soil Science* **26**: 120–125.

TSCHARNTKE, T., GATHMANN, A. and STEFFAN-DEWENTER, I. 1998. Bioindication using trap-nesting bees and wasps and theirnatural enemies: community structure and interactions. *Journal of Applied Ecology* **35**: 708–719.

UNEP (United Nations Environment Programme). 1992. Convention on Biological Diversity*. United Nations. Last updated December 11, 2002, last accessed February 26, 2003. http://www.biodiv.org/convention/articles.asp*

URBINI, A.; SPARVOLI, E. and TURILLAZZI, S. 2006. Social paper wasps as bioindicators: a preliminary research with *Polistes dominulus* (Hymenoptera: Vespidae) as a trace metal accumulator*. Chemosph*. **64**: 697–703.

USEPA. 2002a. Methods for Evaluating Wetland Condition. *Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA-822-R-01-014*.

USEPA. 2002b. Biological indicators of watershed health. *Last updated June 13, 2002, last accessed July 27, 2002. http://www.epa.gov/bioindicators*

VAN JAARSVELD, A. S., FREITAG, S., CHOWN, S. L., MULLER, C., KOCH, S., HULL, H., BELLAMY, C., KRUGER, M., ENDRODY-YOUNGA, S., MANSELL, M. W. and SCHOLTZ, C. H. 1998. Biodiversity assessment and conservation strategies. *Science* **279**: 2106-2108.

VAN STRAALEN, N.M., KRIVOLUTSKII, D. (Eds.), 1996. Bioindicator systems for soil pollution. *NATO ASI Series, Kluwer Academic Publishers*, pp 16.

WAHIZATUL, A. A., LONG, S. H. and AHMAD, A. 2011. Cmposition and distribution of aquatic insect communities in relation to water quality in two fresh wate streams of Hulu Terengganu, Terengganu. *J. Sust. Sc. Manag*. **6** (1): 148-155.

WALTER, G.R., POST, E., CONVERY, P., MENZEL, A., PARMESAN, C., BAIRLEN, F., BEEBEE, T., FOROMONT, J. M. and HOEGHGULDBERG, O. 2002. Ecological responses to recent climate change, *Nature* **416**: 389–395.

WARD, J. V. and STANFORD, J. A. 1979. Ecological factors controlling stream zoo-benthos with emphasis on thermal modifications of regulated streams. *In, Ward, J.V. and Stanford, J. A. (eds) Ecology of regulated streams. Plenum, New York Press*, 35-53.

WARREN, M. S., HILL, J. K., THOMAS, J. A., ASHER, J., FOX, R., HUNTLEY, B., ROY, D. B., TELFER, M. G., JEFFCOATE, S., HARDING, P., JEFFCOATE, G., WILLIS, S. G., GEATOREXDAVIES, J. N., MOSS, D. and THOMAS, C. D. 2001. Rapid responses of British Butterflies to opposing forces of climate and habitat change, *Nature* **414**: 65–69.

WATSON, J. A. L., ARTHINGTON, A. H. and CONRICK, D. L. 1982. Effect of sewage effluent on dragonflies (Odonata) of Bulimba Creek, Brisbane*. Australian Journal of Marine and Freshwater Resources* **33**(3): 517-528.

WENZEL, M., SCHMITT, T., WEITZEL, M. and SEITZ, A. 2006. The severe decline of butterflies on western German calcareous grasslands during the last 30 years: A conservation problem. *Biological Conservation*, **128:** 542-552.

WESTERKAMP, C. and GOTTSBERGER, G. 2000 Diversity pays in crop pollination*. Crop Science*, **40**: 1209-1222.

WHITE, J. and IRVINE, K. 2003. The use of littoral meso-habitats and their macroinvertebrate assemblages in the ecological assessment of lakes. *Aquatic Conservation—Marine and Freshwater Ecosystems* **13**: 331–351.

WILSON R, J., GUTIÉRREZ, D., GUTIÉRREZ, J., MARTÍNEZ, D., AGUADO, R. and. MONTSERRAT, V. J. 2005. Changes to the elevational limits and extent of species ranges associated with climate change, *Ecology Letters* **8**: 1138–1146.

WILSON, E. O. 1988. Biodiversity, *National Academy Press, New York, NY*, pp 400.

WOIWOD, I. and STEWART, A. 1990 Butterflies and moths- migration in the agricultural environment, *In BUNCE, R. and HOWARD, D. E. (Eds.) Species dispersal in agricultural habitats. London, Belhaven Press, pp*. 189–202.

ZIA, A. 2010. Biosystematics of damselflies (Zygoptera: Odonata) of Pakistan, *Ph.D. thesis, PMAS-Arid Agriculture University, Rawalpindi, Pakistan,* pp 135.

ZIA, A., NAEEM, M and HASSAN, S. A. 2008. A list of damselflies (Zygoptera: Odonata) recorded from Azad Jammu & Kashmir, *Pakistan J. Sci. & Indust. Res.,* **51**(1): 329–332.

ZIA, A., RAFI, M. A., HUSSAIN, Z, and NAEEM, M. 2009. Occurrence of Odonata in northern area of Pakistan with seven new records, *Halteres* **1**(1): 48–56.

ZUMA-N ETSHIUKHWI, G., STIGTER, K. and WALKER, S. 2013. Use of Traditional Weather/Climate Knowledge by Farmers in the South-Western Free State of South Africa: Agro-meteorological Learning by Scientists. *Atmosphere* **4**: 383-410.

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PERIONYX SHYAMASREETUS NOV. (CLITELLATA: MEGASCOLECIDAE): A FIRST REPORT FROM INDIA, COLLECTED FROM ODISHA

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ABSTRACT: We report for the first time a new species *Perionyx shyamasreetus* collected from Odisha, Bhubaneswar district (Latitude 20.3119° N and Longitude 85.8606° E) of the state of Orissa in India. This species has been identified to belong to the family of Megascolecidae with 9 genus reported from India and genus, *Perionyx* with 42 species reported from India. The anatomical features include presence of observed elongated body form with a total length of 10.2cm and breadth 3.5 mm. Anal pore is 0.3 mm in diameter. Mouth is 1 mm in diameter. Body colour is observed to be pink in living condition. Colour fades up in preserved state. Total number of body rings is observed to be 157. The structural feature of eye shaped male pores opening between the 18th ring is observed to be unique in this species. This anatomical feature is unique to the species and is not found in any other species of the genus. Female pore obscure. Perichaetine setal arrangement is observed in the body. Racemosal prostate gland is present. This is the first report on this species from India. The anatomical structural peculiarities of this species and its role in evolution remains the major goal of future research.

Key words: *Perionyx shyamasreetus*. nov., Clitellata, Megascolecidae, Odisha

INTRODUCTION

Earthworms belong to the Phylum Annelida and are known as the farmer's friends. The beneficial effect of earthworms in increasing soil fertility has been documented as early as in 1880's since the time of Darwin (DARWIN, 1881). There are more than 6000 species of earthworms reported globally and 561 of them have been reported from India (MANDAL, 2008). They occur in diversified habitats from manure, compost, litter, humus, kitchen drainage, forest land, grassland, agricultural land, plant nursery and play role in enhancing the soil fertility. They are omnivorous, but most of them draw their nutrition from dead and decayed organic matter. In the soil they are exposed to various pathogens, pollutants and polyphenols in plants and have shown properties to survive under harsh conditions. Recently unique presence of drilodefensins has been reported to protect earthworms from the harmful effects of leaf litter plant polyphenols when ingested by them (LIEBEKE *et al.*, 2015). Studies in molecular biology, biochemistry and transcriptome with deep sequencing approaches have enabled the understanding of the robust immune system in earthworms which is significant from the eco-toxicology point of view. For a greater understanding of the robust nature of the earthworm immune system we direct the readers to refer (GHOSH, 2018). The vast number of species and their importance both from the economic and ecotoxicology point of view have enabled them to be important organisms of study. Thus the understanding of their distribution, abundance and ecological condition of the species finds importance. Considerable work on the taxonomic understanding of earthworm is recorded in the study of scientists

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across the globe (BAIRD, 1869; JULKA, 1988; HALDER, 1998; BANDYOPADHYAY *et al*., 2008). This species belongs to family Megascolecidae, which has known genus recorded from India and genus *Perionyx* with 42 species reported from India (HALDER, 1998; BANDYOPADHYAY *et al*., 2008). In this study we report the morphological features that are unique to the new species of earthworm.

MATERIALS AND METHODS

Collection and Identification: The organism named as *Perionyx shyamasreetus* (Fig. 1) was collected in the premises of Bhubaneswar, Odisha, India in moist soil. After making the collection, earthworms were sorted out and cleaned. Then the cleaned earthworms were placed in a tray with a small quantity of water and were slowly killed by anaesthetizing with 70% alcohol by adding drop by drop at frequent intervals. Earthworms usually die in an extended condition by this process. Just after death, the earthworms were kept in 70% alcohol for permanent preservation. The new species was identified in Zoological Survey of India Head Quarter Kolkata, India and was compared with the material of other species available in hand and information on other species is taken from literature.

Dissection: The specimens are kept in 4% formalin for 12 hours prior to dissection that keeps them hard and straight being narcotized in a pot suitable for its length necessary for proper dissection. Earthworm specimens were pinned-up dorsally by two pins on two ends followed by cut from the 5th segment to 23rd segment by sharp blade in keeping the internal organs intact. The specimens were observed under dissection microscopes from Leica microscope. After dissection photograph of main identifying organs like prostate and spermathecal glands were taken. Superficial characters including male and female pores, setal arrangement, Prostomium, clitella region etc. have been studied under microscope.

RESULTS AND DISCUSSION

Family Megascolecidae

The Megascolecidae family includes a large family of the earthworms having native representatives in Australia, New Zealand, Southeast and East Asia, and North America. Distribution of the most ancient lineages of the family may be attributed to the continental drift. Members of this family have the characteristics of megascolecine type of arrangement of male pores, where the vasa deferentia and prostatic ducts unit before opening through a common pore on segment 18. This is unique and different from other related families including Acanthodrilidae, Octochaetidae and Exxidae, where an acanthodriline type of arrangement is observed where the male pores and pores from one or more pairs of prostates open separately near segment 18, but not through a common pore in segment 18. Megascolecidae genera has been identified with either meroic or with multiple nephridia in each segment, or reveal plesiomorphic holoic arrangement of two nephridia per segment or show arrangement comprising of few nephridia, opening into the digestive tract instead of externally opening. Setae arrangement might vary from lumbricine with plesiomorphic or eight per segment or perichaetine with more than eight setae arranged per segment.

According to the article on Burmese earthworm by Gates, 1970 (GATES, 1972), genus under family Megascolecidae and its distribution in and around India, Burma, Srilanka, consisted of (i) *Lampito* with endemic species primarily reported from hilly area of South India, (ii) *Lennoscolex* distributed in southern part of India and Srilanka., (iii) *Perionyx* including, P. *excavatus*, reported from West Indies and Hawaii. (iv) *Pheretima* inclusive of species *alexandri, anomala, bicincta, birmanica, californica, l'diffringens, elongata, hawayana, houlleti, minima, morrisi, papulosa, peguana, planata, posthuma, robusta, rodericensis.*

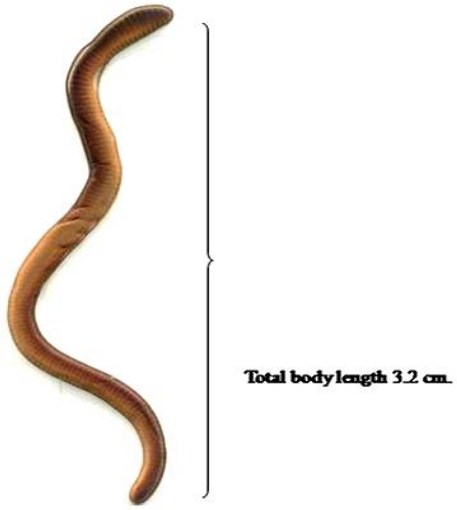

Fig.1

Fig.2A

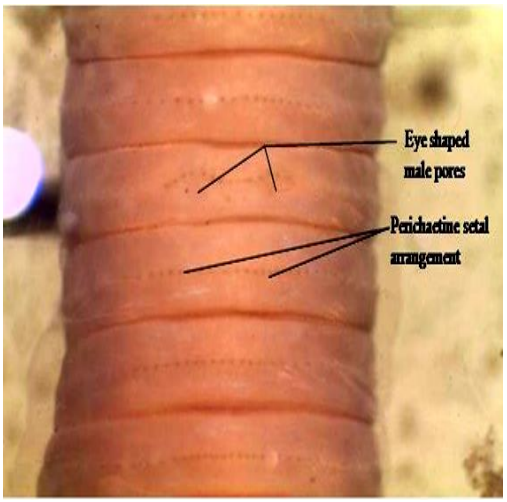

Fig.2B

Fig.2C

Fig. 1: *Perionyx shyamasreetus* full body scan; **Fig.2A:** Epibolic prostomium; **Fig.2B:** Eye-shaped male pore and perichaete in setal arrangement; **Fig.2C: Racemosal prostate glands** characteristic of *Perionyx* genus.

Table 1: Taxonomic Position

Kingdom	Animalia
Phylum	Annelida
Class	Clitellata
SubClass	Oligochaeta
Order	Haplotaxida
Family	Megascolecidae
Genus	***Perionyx***
Species	***shyamasreetus***

Genus *Perionyx*

Perionyx, amongst the megascolecid family of earthworms, have been thought to be evolved in the Indian Peninsula but also migrated to Sri Lanka, Burma probably through the Rajmahal gap, and eastern Himalayas and remains scattered in its distribution bearing a correlation with the presence of organic matter. The heights of the eastern Himalayas with regular rainfall are reported to be the hotspot of evolution of *Perionyx* species (GATES, 1972).

New species: *Perionyx shyamasreetus*

After observation under microscope and dissection the following were observed.

(i) Holotype:

Z.S.I. Reg. No. An 5724/1, 1 ex., Coll. Dr. Shyamasree Ghosh, 23.Xii.2017, Orissa, Bhubaneswar district (20.3119° N ,85.8606° E), Orissa, India.

(ii) Diagnosis (Fig. 1-2):

(i) Morphology: medium and elongated, brownish in colour.
(ii) Ventral region: pinkish in colour.
(iii) Size: The total length of the body is 3.2cm.Breadth 3 mm. Middle part almost half of the body.
(iv) Anal pore: .3 mm.
(v) Number of body rings: Total number of body rings is 157.
(vi) Male pores: Male pores open between the rings 18. Male pore is almost eye shaped (**Fig. 2B**). Small to medium sized.
(vii) Female pore: obscure
(viii) Clitellar region: pinkish white.

(iii) Habit and habitat

The species of earthworms live in the vermicompost garden. It lives in the shadow place and beneath the half superficial lair of the soil. In the month of June -July due to rain it comes out from the soil in search of dry habitat, and favours the process of collection. Breeding season ranges from June –July. It remains embraced together from opposite end at the time of breeding. Male pore is below the female pore which is unique to earthworm and opposite in leeches.

(iv) Distinguishing features

(i) Distribution: Orissa, India.
(ii) Etymology: The specific name is proposed according to the name of the one of the authors who collected the specimen from the Odisha.
(iii) Quadrithecal, pores near mV, at (8/9-9/10)
(iv) Male pores single small
(v) Clitellum not demarcated
(vi) Nephropores, inconspicuous, in one rather irregular rank on each side of the body and near mL.
(vii) First dorsal pore, Between 3 and 4 segment
(viii) Prostomium,epilobous, tongue open.
(ix) Color purple on dorsum, fade up in ventrally
(x) Segments- 157 segments
(xi) Gizzard, V segment
(xii) Esophagus, widened, bead-shaped in xiii and there with calciferous ridges that extend into xiv and xi, valvular in xv.
(xiii) Last hearts, in xii
(xiv) Nephridia avesiculate. Holandric.

(xv) Seminal vesicles, in xi, xii, last pair often continued in pockets of 12/13 back to level of 14/15.
(xvi) Prostates, very small
(xvii) Penial setae, 0.60-0.69 mm, long, 15-25µ thick, ornamented ectally with 6-16 circles of fairly large and elongately triangular spines, tip bluntly rounded or finely pointed or flattened and truncate.
(xviii) Spermathecae, short and small.

The present described species *Perionyx shyamasreetus* exhibits similarity to *Perionyx excavates* (*P. excavatus)* in body shape. It is distinguished from *P. excavatus* in its (i) colour, the clitellar region is observed to be pink in colour (ii) number of rings in the body 157 and (iii) structure of genital pores, (iv) male pores open between the rings 18. Male pore is almost eye shaped (Fig. 2B) small to medium sized is observed to be the unique feature of the species (v) female pore obscure (vi) they occur from small to medium sized with average body length of 10.2 cm and breadth 3.5 mm. (v) anal pore is 0.3 mm. All the species of megascolecidae family are different from the new species of the earthworm. *Perionyx shyamasreetus* is described as a new species from Orissa and is the first report with the unique features including male pores one pair, eye shaped open between the rings 18 is observed to be the unique feature of the species. It is not found in any other species of the genus. They occur from small to medium sized with average body length of 10.2cm and breadth 3.5 mm. anal pore is 0.3 mm. Total number of rings in the body is observed to be 157.The clitellar region is observed to be pink in colour. The anatomical peculiarities (Fig 1-2) observed in the new species remains yet to be studied from evolutionary point view and remains the future scope of the study.

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REFERENCES

BAIRD, W. 1869. Description of a new species of earthworm (*Megascolex diffringens*) found in North Wales. Proceedings of the zoological society of London: 108 Part-**30**: 40-43.

BANDYOPADHYAY, P.K., MANDAL C.K. and MITRA, A.K. 2008. Earthworms of North 24 Parganas, *Rec. Zool. Surv. India:* **108**(Part-3): 21-25.

DARWIN, C. 1881. The formation of vegetable mould through the action of worms with observations on their habits. *Murry, London, 298 pp*.

GATES, G. E. 1972. Burmese Earthworms: An Introduction to the Systematics and Biology of Megadrile Oligochaetes with Special Reference to Southeast Asia. *Transactions of the American Philosophical Society,* **62** (7): 1-326.

GHOSH, S. 2018. Environmental pollutants, pathogens and immune system in earthworms, *Environmental Science and Pollution Research DOI: 10.1007/s11356-017-1167-8*.

HALDER, K.R. 1998. Annelida: Oligochaeta: earthworms, *State Fauna Series 3: Fauna of West Bengal, part* **10**: 17-93.

JULKA, J. M. 1988. Fauna of India & the adjacent Countries: Megadrile Oligochaeta (Earthworms); Haplotaxida: Lumbricina: Megascolecoidea: Octochaetidae, *Zoological Survey of India, Calcutta*, pp 400.

LIEBEKE, M., STRITTMATTER, N., FEARN, S., MORGAN, A. J., KILLE, P., FUCHSER, J., WALLIS, D., PALCHYKOV, V., ROBERTSON, J., LAHIVE, E., SPURGEON, D. J., MCPHAIL, D., TAKÁTS, Z., BUNDY, J. G. 2015. Unique metabolites protect earthworms against plant polyphenols. *Nat. Commun.* **6**: 786-789.

MANDAL, C. K. 2008. Earthworm studies of population in saline and non- Saline soil of Midnapore & Burdwan district of West Bengal. *Rec. Zool. Surv. India.***108** (part-3): 49-54.

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POTENTIALITY OF ERICULTURE FOR POVERTY ALLEVIATION IN DRY LAND AREAS OF TELANGANA – AN ECONOMIC ANALYSIS

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ABSTRACT: Ericulture once confined to the hilly, tribal districts of North Eastern Region of India has spread to several other states including the state of Telangana. The size of land holdings possessed by small and marginal farmers in the state of Telangana is much below the minimum economically viable sizes. Hence, rainfed castor farming in Telangana has been diversified into eri silkworm cocoon production which ensures additional income and family employment during crop leisure period. In this regard, eight castor genotypes were subjected to four defoliation levels *viz*., 30%, 40% and 50% along with control (no defoliation). Significantly no deviation was observed in seed yield from non defoliation plot and 30% defoliated plants. At 50% defoliation on an average, the yield gap was more than 15% in all most all the genotypes. The batch of eri silkworms reared on the leaves of PCH-111 recorded significantly the highest shell yield of 15.5 kg/ha followed by GCH-4 (14.9 kg/ha) and PCH-222 (14.8 kg/ha). If the castor crop is grown for the seed purpose alone, the gross returns and net profit was highest with PCH-111 (Rs. 45,600 and 30,640/-) followed by PCH-222 (Rs. 44,832 and 29,872/-). By utilizing the 30% defoliated leaf for eri silkworm rearing, significantly more gross returns and net profit was recorded with PCH-111 (Rs. 8525 and 6007.5/-), GCH-4 (Rs. 8195 and 5867.5/-) and PCH-222 (Rs. 8140 and 5765/-). That means, these genotypes are suitable both for seed production and eri silkworm rearing. On an average all most all the genotypes recorded Rs. 5,500/- per hectare in addition to the regular seed yield. Significantly higher CB ratio was observed with PCH-111 (1:3.95), PCH-222 (1:3.72) and GCH-4 (1:3.05) while the CB ratio was least with DCH-177 (1:2.32). Hence, from the above results it is very clear that castor plants were able to completely regrow its leaf area when the 30% defoliation occurred during the crop stage without any influence on the seed yield and defoliation beyond this level caused a reduction in the seed yield. That 30% defoliated leaf can be utilized for growing eri silkworms and can get an additional income of Rs. 3000/- acre. However, by utilizing PCH-111, PCH-222 and GCH-4 genotypes farmers can reap higher gross and net returns with more CB ratio.

Key words: Ericulture, defoliation, poverty alleviation, shell yield, CB ratio

INTRODUCTION

Eri is the most popular and rapidly expanding sericulture in the vanya silk map of India and is now getting National as well as International limelight. North East India is rich in Seri biodiversity being a natural abode for a number of sericigenous insects and their host plants (FAO MANUAL, 1987). Ericulture once confined to the hilly, tribal districts of North Eastern Region of India has spread to several other states *viz.,* Andhra Pradesh, Telangana, Madhya Pradesh, Tamil Nadu, Karnataka, Maharashtra, Uttarakhand, Uttar Pradesh, Jharkhand, Bihar, West Bengal, Odisha, Punjab, Uttarakhand, Chhattisgarh, Maharashtra, Gujarat, Rajasthan etc. (SAHU *et al.,* 2006). The reason being, eri silkworm, *Samia cynthia ricini* Boisduval is a multivoltine and polyphagous species and it can be reared throughout the year depending on the availability of feed (THANGAVELU AND PHUKON, 1983; DEBARAJ *et al.,* 2003; CHOWDHURY, 1982; KRISHNASWAMI *et al.,* 1971 and KAPIL, 1967).

The size of land holdings possessed by small and marginal farmers in the state of Telangana is much below the minimum economically viable sizes, earning income almost equal to that of agricultural labourers. The income obtained from cultivation of agricultural crops in one hectare of land is not sufficient to cross the poverty line. In Telangana out of 55.54 lakh farm holdings, 34.41 lakhs are marginal farmers and 13.27 lakh farmers are small farmers (2016-17). Hence diversification of agriculture into various income fetching enterprises has become the order of the day. Rainfed castor farming in Telangana has been diversified into eri silkworm cocoon production which ensures additional income and family employment during crop leisure period. The state has successfully utilized 30-40% of castor leaves for obtaining additional income through eri silkworm rearing without impairing castor seed yield (TEOTIA *et al.,* 2003). Further, it is an off-farm activity that ensures gainful family employment with 55% women participation (JAYA PRAKASH *et al.,* 2005; 2006). Cultivating these crops solely for the purpose of eri silkworm rearing may not be economical and practical due to high cultivation cost. But when a portion of the total foliage can be harvested without affecting the main produce *viz.,* castor seed, it may be economical and can contribute towards economic independence of farmers (DEVAIAH *et al.,* 1984). In this regard, present experiment was planned to know the effect of different defoliation levels on the seed yield and its effect on economics of dry land farmers of Telangana.

MATERIAL AND METHODS

The experiment was conducted at the research farm of Regional Agricultural Research Station, Palem for three seasons *viz., Rabi* 2011-12, *Kharif* 2012-13 and *Rabi* 2012-13 to study the effect of defoliation on seed yield and additional income we can generate by rearing eri silkworms. The experiment was conducted in randomized block design with eight genotypes of castor *viz.,* Haritha, Kranthi, Kiran, DPC-9, PCH-111, PCH-222, GCH-4 and DCH-177 replicated thrice. Treatments consisted of four defoliation levels like no defoliation, 30%, 40% and 50%. The plants were hand defoliated at 45, 95 and 135 DAE so as to synchronize with eri silkworm rearing cycle. Removed leaf yield from different defoliation levels and total seed yield was quantified. The crop received recommended dose of fertilizers and the crop was supplemented with 20 kg of urea and 10 kg of muriate of potash per acre after every defoliation.

Eri silkworm rearing was conducted in ericulture laboratory of RARS by following the standard rearing methods adopted by DAYASHANKAR, 1982. Cost of eri silkworm rearing per 100 dfls, number of eri dfls reared per hectare, eri shell yield was calculated. Similarly cost of cultivation, seed yield/ha were recorded. The pooled data of three years including the seed yield at different defoliation levels and the deviation in seed yield were recorded. The economics pertaining to castor cultivation and eri silkworm rearing were computed separately so as to calculate the additional income generated through ericulture. The yield components were subjected to statistical analysis following one way ANOVA.

RESULTS AND DISCUSSION

Defoliation per cent vs seed yield: To know the effect of defoliation on seed yield, leaf number removed ranged from 3 to 8, 4 to 12 and 5 to 15 at 30, 40 and 50% defoliation levels. Exact defoliation levels could not be imposed as definite number of whole leaves was removed. On an average 28, 39 and 52% leaf area was removed during three seasons from 30, 40 and 50% defoliation, respectively. As evident from figure 1, significantly no deviation was observed in seed yield from non defoliation plot and 30% defoliated plants. With 40% defoliation more than 5% yield loss was observed in DCH-177, GCH-4 and PCH-222 genotypes, while with 50% defoliation, the highest yield gap of 20% was observed in DCH-177, while it was 18 and 17% in Kranthi and DPC-9 respectively. On an average, the yield gap was more than 15% in all most all the

genotypes at 50% defoliation (Fig.-1). A disturbance such as defoliation can influence several factors on the plant physiology like sink and source equilibrium, production of hormones and change in the use of light by canopy layers. In theory, the loss of any leaf causes a reduction of the photosynthesizing area, a waste of carbohydrates stored on that lost structure and an increase in the demand of energy for regrowth of leaves. After defoliation, a plant can recover without significant impact on productivity, but it is expected that a progressive increase in such damage would lead to increase loss of productivity (ENDAN *et al.,* 2006).

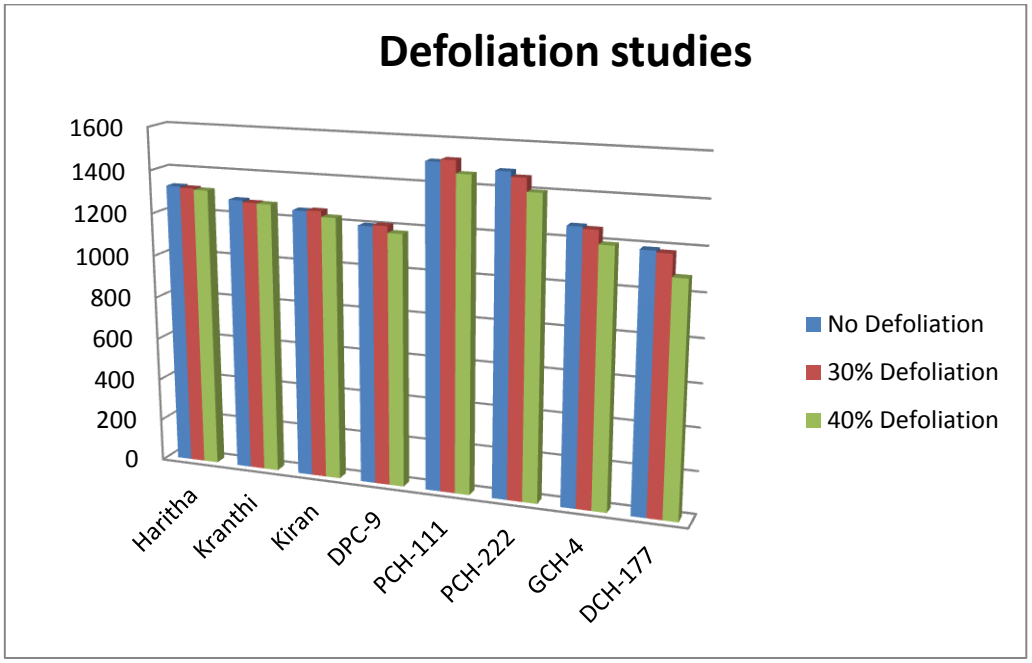

Fig-1: Defoliation per cent v/s seed yield among selected castor genotypes
(Pooled data of *Rabi* 2011-12; *Kharif* 2012-13 and *Rabi* 2012-13)

When castor plants were defoliated in the vegetative stage (on/before 45 DAE), they were able to replace all the leaf area lost in a short period of time and apparently the defoliation in this phase had no impact on the plant. Indeed, there are several studies with crops like soybean, peanut, wheat and sunflower concluding that one event of defoliation on initial stages of vegetative growth had no impact, or in some cases a positive effect on yields (GAZZONI AND MOSCARDI, 1998; SHAFIULLAH *et al.,* 2000; ENDAN *et al.,* 2006; AHMADI AND JOUDI, 2007), although, yield reductions are related when two sequential defoliations were applied (REICHERT AND COSTA, 2003). RAMESH AND PRASAD, (2001) conducted the defoliation studies in Aruna variety of castor. The results showed that removal of leaves proximal to main spike significantly affected seed yield. Defoliation and shading appear to decide the pattern of assimilate distribution in plants (KING *et al.,* 1967). The reduction in yield due to defoliation implies that all leaves on the plant contribute to seed yield. Removal of any leaf decreases the yield. REDDY *et al.,* (1989) reported that maximum dry matter production in cultivar Aruna is achieved at optimum leaf area index of 0.85 and 0.62 at 90 DAS and at maturity, respectively (RAMESH AND PRASAD, 2001).

Appendix-I: Cost of castor cultivation under recommended practices (per ha) (Pooled data of *Rabi* 2011-12; *Kharif* 2012-13 and *Rabi* 2012-13)

S. No.	Particulars	Quantity/Unit	Amount (Rs.)
1.	Land Preparation		
	a) Tractor	3 hrs	1800
	b) Bullock pair	1 BPD	800
2.	Farm yard manure		1700
3.	Seed Cost	10 kgs	700
4.	Sowing		
	a) Bullock pair	2 BPD	1600
	b) Human labour	8 MD	1280
5.	Fertilizers	40:40:0	2200
6.	Intercultural operations	8 MD	1280
7.	Non-recurring expenditure		800
8.	Harvesting and Processing	15 MD	2400
		Total	14,560

BPD – Bullock pair days; MD – Man days

Appendix-II: Cost of Eri silkworm rearing (per 100 Dfls)

S. No.	Particulars	Amount (Rs.)
1.	Cost of Dfls	200
2.	Cost of disinfectants	250
3.	Non-recurring expenditure	300
4.	Depreciation value on building	-
5.	Cost of Cocoon transportation and marketing	200
	Total	950

Castor cost of cultivation: The details pertaining to cost of castor cultivation was furnished in Appendix I. The cost of castor cultivation pertaining to varieties is Rs. 14,560/-, while for hybrids the cultivation cost is Rs. 14,960/- the only difference is pertaining to seed cost. Significantly highest seed yield was recorded with PCH-111 (1425 kg/ha), PCH-222 (1401kg/ha) closely followed by GCH-4 (1305 kg/ha), accordingly gross returns and net profit was high with these genotypes. The CB ratio based on cost of castor cultivation is more with PCH-111 (1:2.01) followed by PCH-222 (1:1.92) (Table-1).

Cost of eri silkworm rearing: Cost pertaining to eri silkworm rearing for 100 dfls is shown in Appendix II. On compiling the pooled data of three seasons, cost of eri silkworm rearing was significantly more with PCH-111 (Rs. 2517.5/-) followed by PCH-222 (Rs. 2375/-) as the genotypes accounted for more number of eri layings per hectare i.e 265 and 250 respectively. However, the cost of eri silkworm rearing was less with DCH-177 (Rs. 1710/-), which accounted for least number of eri layings reared per hectare (180) (Table- 2). JAYARAMAIAH AND CHINNASWAMY, (1998) estimated that 200 eri dfls can be reared from the leaf obtained from one ha with 25% defoliation, which fetches an additional income of Rs. 3000/-

Table-1: Cost of production of castor seed per hectare among selected castor genotypes (Pooled data of *Rabi* 2011-12; *Kharif* 2012-13 and *Rabi* 2012-13)

Genotype	Cost of castor cultivation	Seed Yield (Kg)*	Gross Returns (Rs.)	Net profit (Rs.)	C:B Ratio
Haritha	14,560	1298	41,536	26,976	1:1.75
Kranthi	14,560	1250	40,000	25,440	1:1.62
Kiran	14,560	1245	39,840	25,280	1:1.60
DPC-9	14,560	1224	39,168	24,608	1:1.52
PCH-111	14,960	1425	45,600	30,640	1:2.01
PCH-222	14,960	1401	44,832	29,872	1:1.92
GCH-4	14,960	1305	41,760	26,800	1:1.85
DCH-177	14,960	1194	38,208	23,248	1:1.50
F-test	-	Sig	Sig	Sig	-
CD at 5%	-	135.49	202.91	134.18	-
SEM ±	-	42.50	134.28	98.64	-

*Castor seed cost – Rs. 32/Kg

Table-2: Cost of production of eri cocoons per hectare among selected castor genotypes (Pooled data of *Rabi* 2011-12; *Kharif* 2012-13 and *Rabi* 2012-13)

Genotype	No. of Eri Dfls reared	Cost of Eri silkworm rearing (Rs.)	Eri Shell Yield (Kg)**	Gross Returns (Rs.)	Net profit (Rs.)
Haritha	202	1919	13.5	7425	5506
Kranthi	200	1900	12.5	6875	4975
Kiran	192	1824	12.0	6600	4776
DPC-9	185	1757.5	11.8	6490	4732.5
PCH-111	265	2517.5	15.5	8525	6007.5
PCH-222	250	2375	14.8	8140	5765
GCH-4	245	2327.5	14.9	8195	5867.5
DCH-177	180	1710	11.9	6545	4835
F-test	Sig	Sig	Sig	Sig	Sig
CD at 5%	13.21	38.61	0.18	42.62	32.64
SEM ±	1.254	7.421	0.072	12.61	9.16

**Cost of eri cocoon shell – Rs. 550/kg

Eri shell yield: All the genotypes exerted significant influence on the shell yield. The batch of eri silkworms reared on the leaves of PCH-111 recorded significantly highest shell yield of 15.5 kg/ha followed by GCH-4 (14.9 kg/ha) and PCH-222 (14.8 kg/ha). These results are inconformity with LAKSHMI NARAYANAMMA *et al.,* 2013, who reported that the cocoons spun by the worms fed on PCH-111 and GCH-4 genotypes recorded significantly superior shell weight. Significantly lowest shell yield was recorded by the eri silkworms reared with the leaves of DPC-9 (11.8 kg/ha) (Table 3). SANNAPPA and JAYARAMAIAH (1999) and DEVAIAH AND DAYASHANKAR (1982) opined that the shell weight varied with type of hosts provided at the larval stage.

Gross returns and net profit: If the castor crop is grown for the seed purpose alone, the gross returns and net profit was highest with PCH-111 (Rs. 45,600 and 30,640/-) followed by PCH-222 (Rs. 44,832 and 29,872/-) (Table-1). By utilizing the 30% defoliated leaf for eri silkworm rearing, significantly more gross returns and net profit was recorded with

PCH-111 (Rs. 8525 and 6007.5/-), GCH-4 (Rs. 8195 and 5867.5/-) and PCH-222 (Rs. 8140 and 5765/-) (Table-2). That means, these genotypes are suitable both for seed production and eri silkworm rearing. On an average all most all the genotypes recorded Rs. 5500/- per hectare in addition to the regular seed yield. These results are inconformity with the results of MISRA (1999), who reported that when castor was grown only for seed production the net profit was Rs. 2345/- per ha, while the net profit was Rs. 5406/- when grown for both seed cum eri cocoon production. Further, MISRA (2001) reported 16% net profit when castor was used for seed production and 34% when used for eri cocoon production. SURYANARAYANA *et al.* (2003) obtained net income of Rs. 11,105/- per acre per year through eri silkworm rearing. On the other hand, PANDEY (2003) could get net income of Rs. 3000 per acre during first year from Ericulture and it was Rs. 13,256 from second year onwards. This variation existed in economics of castor seed cum eri cocoon production might be attributed to the difference in input and output prices, which vary from time to time.

CB Ratio: Marked differences in the net cost: benefit ratio was observed among castor genotypes when they were used both for seed and eri cocoon production. Significantly higher CB ratio was observed with PCH-111 (1:3.95), PCH-222 (1:3.72) and GCH-4 (1:3.05) while the CB ratio was least with DCH-177 (1:2.32) (Table-3). CHANDRAPPA *et al. (*2003) reported high CB ratio with JI-226 genotype when used both for castor seed and eri cocoon production. SINGH *et al.* (2014) realized high CB ratio, which revealed that ericulture is a profitable venture for the poor and marginal farmers of North East India. However, there is an enormous scope for ericulture in castor growing areas without hampering castor seed production and it also provides a supportive economy for the small and marginal farmers. It is remarkable for its low investment, high returns which make it as a profitable venture and an ideal agro based industry for castor growers of Telangana region.

Table-3: Cost of production of eri cocoons per hectare among selected castor genotypes (Pooled data of *Rabi* 2011-12; *Kharif* 2012-13 and *Rabi* 2012-13)

Genotype	**Cost of cultivation (Rs.)**	**Gross Returns (Rs.)**	**Net profit (Rs.)**	**C:B Ratio**
Haritha	16,479	48961	32482	1:3.01
Kranthi	16460	46875	30415	1:2.84
Kiran	16384	46440	30056	1:2.82
DPC-9	16317.5	45658	29340.5	1:2.54
PCH-111	17477.5	54125	36647.5	1:3.95
PCH-222	17335	52972	35637	1:3.72
GCH-4	17287.5	49955	32667.5	1:3.05
DCH-177	16670	44753	28083	1:2.32
F-test	-	Sig	Sig	-
CD at 5%	-	465.98	301.18	-
SEM ±	-	192.81	185.64	-

From the above results it is very clear that castor plants were able to completely re grow its leaf area when the 30% defoliation occurred during the crop stage without any influence on the seed yield and defoliation beyond this level cause a reduction in the seed yield. That 30% defoliated leaf can be utilized for growing eri silkworms and can get an additional income of Rs. 3000/- acre. However, by utilizing PCH-111, PCH-222 and GCH-4 genotypes farmers can reap higher gross and net returns with more CB ratio. The

concept of eri silkworm rearing is to fetch additional income and generate gainful employment among rural populace by utilizing 30% of the unwanted foliage from host crops grown under rain fed conditions of Telangana. Eri silkworm rearing, being a subordinate crop, is considered as mere source of additional income and is in no way comparable with the returns of mulberry sericulture. LAKSHMANAN *et al.* (1996) observed that there were intangible returns from sericulture; however, the cost benefit ratio with rise in net returns was at higher side. The same trend may not last forever, as the cocoon price is totally determined by demand and supply of the product.

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REFERENCES

AHMADI, A. and JOUDI, M. 2007. Effects of timing and defoliation intensity on growth yield and gas exchange rate of wheat grown under well-watered and drought conditions. *Pakistan Journal of Biological Sciences* **10**: 3794–3800.

CHANDRAPPA, D., SANNAPPA, B., RAMAKRISHNA NAIKA, MANJUNATH, K. G. and GOVINDAN, R. 2003. Differential performance of castor genotypes on seed yield and eri cocoon production and their economic analysis under rain fed conditions. *Global Journal of Biology, Agriculture and Health Sciences* **1**(2): 1-3.

CHOWDHURY, S. N. 1982. Eri silk industry. *Directorate of Sericulture and Weaving, Govt. of Assam, Guwahati.*

DAYASHANKAR, K. N. 1982. Performance of eri silkworm, *Samia cynthia ricini* Boisduval on different host plants and economics of rearing on castor under Dharwad conditions. *M. Sc. (Ag) Thesis, UAS, Bangalore* P. 86.

DEBARAJ, Y., DATTA, R. N., DAS, P. K. and BENCHAMIN, K. V. 2003. Eri silkworm crop improvement – A review. *Indian Journal of Sericulture* **41**: 100-105.

DEVAIAH, M. C. and DAYASHANKAR, K. N. 1982. Effect of different host plants on the economic traits of eri silkworm, *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae). *National Seminar on Silk Research and Development*, *CSB, March 10-13, Bangalore*, 152.

DEVAIAH, M. C., GOVINDAN, R. and THIPPESWAMY, C. 1984. Economics of rearing eri silkworm *Samia cynthia ricini* Boisduval on castor, *Ricinus communis* L. *Indian Journal of Sericulture* **23**: 117-120.

ENDAN, J. B., YOUNG, J. H. and AWAL, M. A. 2006. Effect of hand defoliation on peanut growth. *Asian Journal of Plant Sciences* **5**: 281-286.

FAO, 1987. Manual on sericulture. *Central Silk Board, Bangalore*.

GAZZONI, D. L. and MOSCARDI, F. 1998. Effect of defoliation levels on recovery of leaf area, on yield and agronomic traits of soybeans. *Pesqui. Agropeau. Bras.* **33**: 411-424.

JAYA PRAKASH, P., JAIKISHAN SINGH, R. S., SANJEEVA RAO, B. V. and SURYANARAYANA, N. 2006. Rearing performance of Eri silkworm (*Samia cynthia ricini* Donovan) on castor and tapioca in non-traditional areas. *Paper presented in the National workshop on Eri food plant, Central Muga and Eri Research & Training Institute, Central Silk Board, Lahdoigarh (Assam), Guwahati, October 11-12, 2006*, Pp. 38-49.

JAYA PRAKASH, P., SASIKALA, A. and SURYANARAYANA, N. 2005. Ericulture – A tool for rural women development in Andhra Pradesh, India. *Proceedings of XX Congress of International Sericulture Commission, Vol. II(3), International Sericulture Commission, France and Central Silk Board, Bangalore, India, December 15-18, 2005*, pp. 106-113.

JAYARAMAIAH, M. and CHINNASWAMY, K. P. 1998. Integration of ericulture for sustenance of agriculture production systems in Karnataka. *The third International Conference on Wild Silk moths*, *11-14 November, Bhubaneswar*, 110.

KAPIL, R. P. 1967. Effects of feeding different host plants on the growth of larvae and weight of cocoons of *Philosamia ricini* Hutt. *Indian Journal of Entomology* **29**: 295-296.

KING, R. W., WARDLAW, I. F. and EVANS, L. T. 1967. Effect of assimilate utilization on photosynthetic rate in wheat. *Planta* **77**: 261-276.

KRISHNASWAMI, S., MADHAVA RAO, N. R., SURYANARAYANA, S. K. and SUNDARAMURTHY, T. S. 1971. Sericulture Manual-3, Silk Reeling, *FAO, Rome* Pp. 112.

LAKSHMANAN, S., MALLIKARJNA, B., JAYARAM, H., GANAPATHY RAO, R., SUBRAMANIAM, M. R., GEETHA DEVI, R. G. and DATTA, R. K. 1996. Economic issues of production of mulberry cocoon in Tamil Nadu – A micro Economic Study. *Indian Journal of Sericulture* **35**(2): 128-131.

LAKSHMI NARAYANAMMA, V., DHARMA REDDY, K. and VISHNUVARDHAN REDDY, A. 2013. Castor genotypes on rearing and cocoon parameters of eri silkworm, *Samia cynthia ricini*. *Indian Journal of Plant Protection* **41**(2): 127-131.

MISRA, S. D. 1999. ARUNA variety, a unique host for the eri silkworm. Part I. Two revenues from the same resource. *Indian Journal of Sericulture* **38**(2): 95-101.

MISRA, S. D. 2001. Aruna variety, a unique host for the eri silkworm. Part 2. Ericulture for stressed ecosystem. *Indian Journal of Sericulture* **40**(1): 21-26.

PANDEY, R. K. 2003. Ericulture in Uttar Pradesh. *Indian Silk* **41**(12): 21-24.

RAMESH, T. and PRASAD, M. M. K. D. 2001. Effect of source manipulation on yield of castor *(Ricinus communis* L.) *Madras Agricultural Journal* **87**(10/12): 661-663.

REDDY, D. N. R., KOTIKAL, Y. K. and VIJAYENDRA, M. 1989. Development and silk yield of Eri silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae) as influenced by the food plants. *Mysore Journal of Agricultural Sciences* **23**: 506-508.

REICHERT, J. L. and COST, E. C. 2003. Desfolhamentos continuos e sequenciasis simulando danos de pragas sobre a cultivar de soja BRS 137. *Clinical Rural* 33: 1-6.

SAHU, M. N., BHUYAN. and DAS, P. K. 2006. Eri silkworm, *Samia ricini* (Lepidoptera: Saturniidae) Donovan, Seed production during summer in Assam. *In: Proceeding of Regional seminar on "Prospects and problems of sericulture as an economic enterprise in North West India, Dehradune, India,* pp: 490-493.

SANNAPPA, B. and JAYARAMAIAH, M. 1999. Interaction of Castor genotypes and breeds of Eri silkworm in relation to cocoon and grainage parameters. *Mysore Journal of Agricultural Sciences* **33**: 214-223.

SHAFIULLAH, M. A., KHAN, M. A., POSWAL, M. A. and RANA, B. 2000. Simulation of leaf damage by artificial defoliation and its effect on sunflower (*Helianthus anuus* L.) performance. *Pakistan Journal of Biological Sciences* **3**: 459-462.

SINGH, R., HANDIQUE, P. K. and SONOWAL, P. 2014. Techno economic feasibility of ericulture in the plains of North East India. *Biological Forum- An International Journal* **6**(1): 44-47.

SURYANARAYANA, N., DAS, P. K., SAHU, A. K., SARMA, M. C. and PHUKAN, J. D. 2003. Recent advances in ericulture. *Indian Silk* **4**: 5-12.

TEOTIA, R. S., SATYANARAYANA, K., RAJASHEKAR, K., GOEL, R. K. and KRISHNA RAO, J. V. 2003. UNDP assistance in development of Ericulture. *Indian Silk,* **41**(14): 13-18.

THANGAVELU, K. and PHUKON, J. C. 1983. Food Preference of Eri Silkworm *Philosamia ricini* Hutt (Saturnidae: Lepidoptera). *Entomon,* **8**: 311-315.

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FISH DIVERSITY OF AAYIRAMTHENGU MANGROVE OF KAYAMKULAM ESTUARY, KERALA

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ABSTRACT: The status of fish diversity and seasonal variation in their distribution and abundance were investigated in Aayiramthengu region of Kayamkulam Backwater. In total 21 samples have been analyzed which were taken throughout June 2016-May 2017. The major objective of this study was to find out the diversity of fishes in the Aayiramthengu region of Kayamkulam estuary, Kerala. From the present study 17 fish species were recorded and monthly diversity indices were calculated. The determined diversity indices are Species richness, Shannon index, Simpson's index, Margalef index, Evenness index and Dominance index. The lowest Margelef'S index (1.077-2.6) was noticed in the month of May and highest in the month of August. In the case of Evenness (0.654-0.884),highest in the month of October and lowest in June. Dominance index was recorded in the range between 0.126-0.299, lowest value in March and highest value in July. From the present study higher diversity values in monsoon and post monsoon season and lower diversity values showed in premonsoon season.

Key words: Icthyofauna, Biodiversity, IUCN, Diversity indices.

INTRODUCTION

The backwaters of Kerala have a significant role in the socio- economic and cultural history of the state. Estuaries are the meeting place of fresh water from rivers and salt water from the sea and as such are dynamic environments characterized by large fluctuations in environmental conditions (JAMES *et al*., 2007). Biodiversity refers to the abundance and the variety within and among fauna and flora as well as the ecosystem and ecological processes to which they belong and is thus usually considered at ecosystem, species and genetic levels (BHARATHA LAKSHMI *et al*., 2017). The species diversity of an ecosystem is related to the amount of living and non living organic matter present in it. Fishes are one of the important elements in the economy of many nations as they have been a stable item in the diet of many people (KURUP and SAMUEL, 1985). Using species assessment as a tool is one way of understanding the threats to biodiversity ecosystem and specially the impacts of changing ecosystem on human well being. However, considering the reason mentioned above present study aimed to describe fin fish assemblages structure at Kayamkulam Backwater.

MATERIALS AND METHODS

Kerala lies towards the South west coast of India, Aayiramthengu is a coastal region located in Kollam District (9^0 54'41.96'' N and 76^0 18' 32.36"E) east of Kayamkulam estuary which opens to the Arabian Sea. Cast net, gill net and drag net were used for fish sampling on monthly basis. After sorting and counting, representative fish fauna were identified upto species level (DAY, 1889; TALWAR and JHINGARAN 1991; JAYARAM, 2010). Total numbers for fishes were recorded on monthly data from the Aayiramthengu region. Seasonal species abundance data used as input data for the calculation of biodiversity indices such as Dominance (D), Evenness, Shannon-Weiner(H'), Simpson'Index(S), and Margalef's('d') indices by using PAST 3.1 software.

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RESULTS AND DISCUSSION

The primary aim of the present study was to find out the fish diversity of the Aayiramthengu Mangrove region of the Kayamkulam Estuary. Distinct variations in distribution and abundance of fish biodiversity in the Aayiramthengu region were observed during the present study. Abundance of fin fish was tremendously high during the monsoon season and post monsoon due to the reason of heavy rain. Total number of fish species was encountered during the study period from the study area comprising 19 species of fin fishes belonging to 12 families, 6 orders and 16 genera (Table-1). From the present study shows that the diversity decreased from post monsoon (Oct-Jan) to the premonsoon period (Feb-May) after that increasing the diversity during the monsoon period(Jun-Sep). Distribution and abundance of fish diversity in different estuaries in Kerala has been extensively studied by MOGALEKAR *et al*. (2015); KURUP and SAMUEL (1987); KURUP *et al.* (1989); HARIKRISHNAN *et al.* (2011); BIJOY NANDAN *et al.* (2012) and REMYA and AMINA (2018) reported 125 fin fishes belonging to 13 orders, 87 genera and 57 families from the Kayamkulam Backwater.

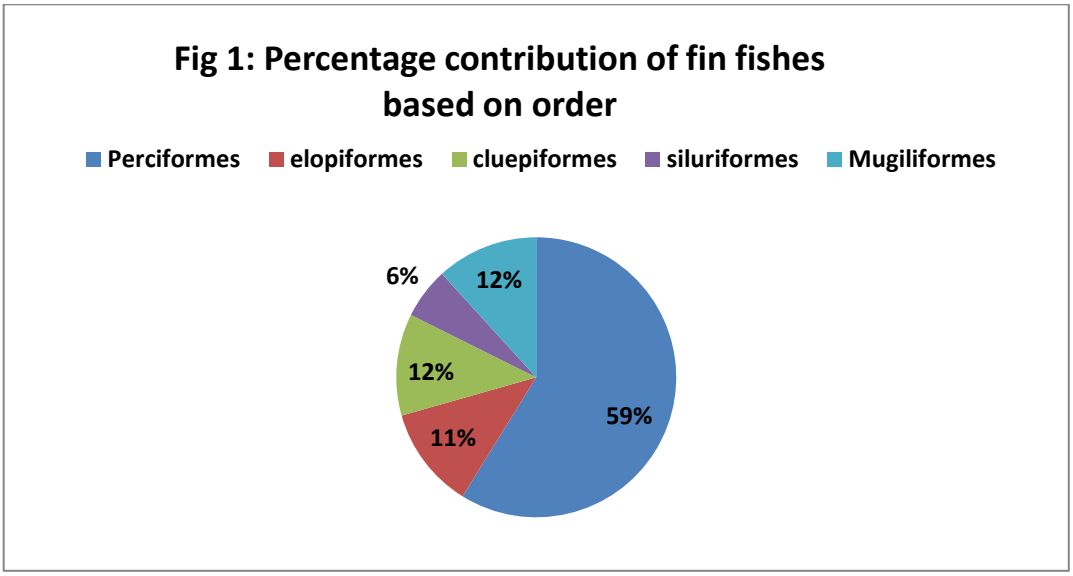
Table-1: Piscine taxonomy of fishes collected from Aayiramthengu region of Kayamkulam Estuary

S. No	Order	Family	Scietific names	IUCN Status
1	Perciformes	Ambassidae	*Ambassis ambassis*	NE
2	Perciformes	Carangidae	*Caranx ignobilis*	LC
3	Perciformes	Carangidae	*Caranx sexfasciatus*	NE
4	Clupeiformes	Clupeidae	*Escualosa thoracata*	NE
5	Elopiformes	Elopidae	*Elops machnata*	NE
6	Perciformes	Cichlidae	*Etroplus maculates*	NE
7	Perciformes	Cichlidae	*Etroplus suratensis*	NE
8	Perciformes	Gerreidae	*Gerres oyena*	NE
9	Perciformes	Gerreidae	*Gerres filamentosus*	NE
10	Perciformes	Gobidae	*Glossogobius giuris*	NE
11	Perciformes	Leiognathidae	*Gazza minuta*	NE
12	Perciformes	Leiognathidae	*Leiognathus brevirostris*	NE
13	Mugiliformes	Mugilidae	*Liza parsia*	NE
14	Elopiformes	Megalopidae	*Megalops cyprinoides*	NE
15	Siluriformes	Bagridae	*Mystus gulio*	NE
16	Mugiliformes	Mugilidae	*Mugil cephalus*	NE
17	Clupeiformes	Clupeidae	*Nematolosa nasus*	NE

NE: Not evaluated, LC: Least Concern

Biodiversity indices

Various diversity indices were calculated and presented in Table-2. The Shannon-Weiner index (1.45-2.229) and Simpson's index (0.7-0.87) were minimum in the month of March during the pre-monsoon season and maximum recorded in the month of July during the monsoon season. The lowest Margelef'S index (1.077-2.6) was noticed in the month of May during the premonsoon season and highest in the month of August during the monsoon season. In the case of Evenness (0.654-0.884), highest in the month of October during the post monsoon and lowest in June during the monsoon season.



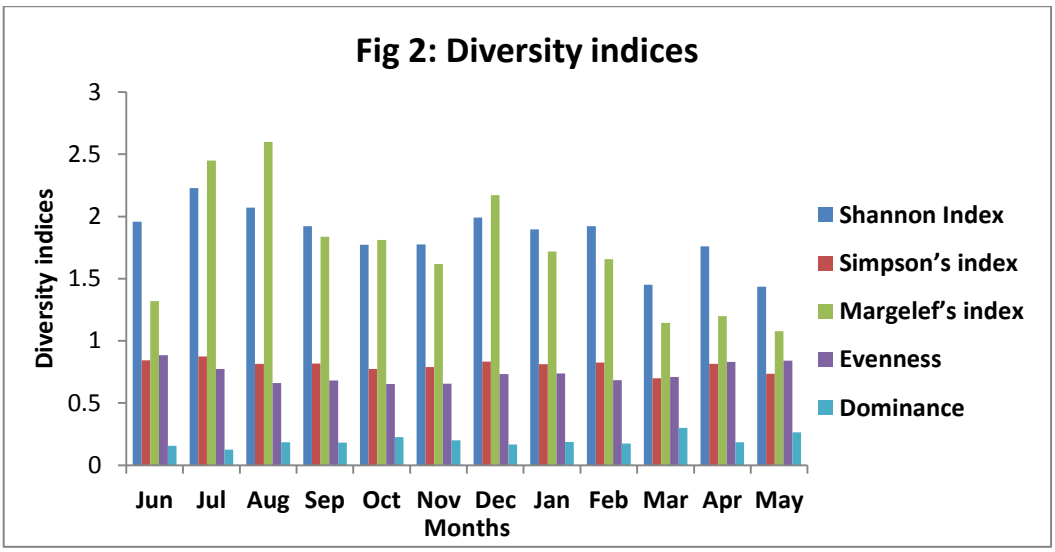


Table-2: Diversity indices of fishes from Aayiramthengu Mangrove of Kayamkulam Estuary during Feb 2016-Jan 2017

Months	Shannon Index	Simpson's index	Margelef's index	Evenness	Dominance
June	1.957	0.844	1.319	0.884	0.155
July	2.229	0.8731	2.45	0.774	0.126
August	2.07	0.815	2.6	0.66	0.184
September	1.921	0.818	1.838	0.682	0.181
October	1.773	0.774	1.81	0.654	0.225
November	1.775	0.79	1.617	0.655	0.2

Contd… Table-2

Table-2. Contd…

Months	Shannon Index	Simpson's index	Margelef's index	Evenness	Dominance
December	1.992	0.833	2.172	0.732	0.167
January	1.896	0.812	1.719	0.739	0.187
February	1.922	0.826	1.656	0.683	0.173
March	1.45	0.7	1.144	0.71	0.299
April	1.76	0.815	1.199	0.83	0.184
May	1.436	0.735	1.077	0.841	0.264

Dominance index was recorded in the range between 0.126-0.299, lowest value in March and highest value in July. From the present study higher diversity values in monsoon and post monsoon season and lower diversity values showed in premonsoon season. In a healthy environment, due to rich faunal assemblages, the total phylogenetic diversity is always higher (AJMAL KHAN, 2008). Fig. 2 shows the graphical representation of the calculated fish diversity indices in Aayiramthengu Mangrove region of the Kayamkulam estuary, recording high fish diversity indices noticed in monsoon season and low diversity in premonsoon period. Diversity involves both the species richness and species evenness.

REFERENCES

ALI, A., RAGHAVAN, R. AND DAHANUKAR, N. 2011. *Puntius denisonii*. *In: IUCN Red List of Threatened Species. Version 2011. 1. www.iucnredlist.org. Downloaded on 24th June 2011*

AJMAL KHAN, S. 2008. Statistical Packares for Assessing Biodiversity. *Winter School on Advanced Biological Techniques For Fisheries.Professionals*,103-113.

DAY, F. 1889.The Fauna of British India, Including, Ceylon and Burma, *Fishes.Taylor and Francis, London*. **1**(2): 548

EASA, P. S. and SHAJI, C. P. 1995. Fresh water fish diversity in Kerala part of the Nilagiri biosphere reserve. *Research report. Peechi. Kerala Forest Research Institute*, 1995.

IUCN (2011). *IUCN Red list of Threatened Species. version 2011.1. <http/www.iucnredlist.org> (Accessed 12.06.2012).*

JAYARAM, K. C. 1999. The fresh water fishes of the Indian Region, *Narendra Publishing House. Delhi*, pp 551.

MOGALEKAE, H. S., ANSAR, C. P., RAMAN, N. N., JAYACHANDRAN, K. V., DINESH, K. and KOLHE, S. 2015. Fish diversity of Vembanad Lake in the Panangad-Kumbalam region of Kochi, Kerala, India. *Poll Res*. **34**(2): 345-349.

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EFFICACY OF BOTANICALS AS SEED PROTECTANTS ON THE INFESTATION AND BIOLOGY OF PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* LINN.

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ABSTRACT: Studies were conducted during 2014-15 to evaluate the efficacy of botanical molecules as their seed treatment (ST) in greengram and blackgram against pulse beetle, *Callosobruchus chienensis* (L.) infestation under ambient storage conditions. The experiment was laid out in CRD with 6 treatments including untreated control and replicated four times. The botanicals are begonia, naguari, curry leaf powder@10g/kg pulse seed, citronella oil @10 ml and sweet flag formulation 6EC @ 10 ml/kg seed were applied as seed treatment. The results revealed that bruchid mortality indicate no mortality in untreated control as against 45.0 to 75.5% mortality due to botanicals treatments following 7 days exposure period after six months of seed treatment of blackgram and greengram. Less fecundity was recorded in ST with sweet flag formulation both in greengram (8.0 eggs) and blackgram (8.5 eggs). The seed treatment with sweet flag formulation registered lowest adult emergence in greengram (2.75-7.25 adults and blackgram (1.75- 6.25 adults). Highest reduction of seed damage on control was estimated in sweet flag formulation (82.69) in greengram and begonia leaf powder (86.96) in blackgram. The results suggested that the botanicals i.e. sweet flag formulation, begonia leaf powder and citronella oil were found most effective seed protectant in pulses over others and have great potentiality in suppressing the seed infestation by the bruchid to the minimum level with appreciably no adverse effect on seed viability upto 6 months of storage.

Key words: Pulse beetle, *Callosobruchus chinensis*, greengram, blackgram, botanicals

INTRODUCTION

Pulse beetle, *Callosobruchus chinensis* (L.) (Bruchidae: Coleoptera) is the most wide spread and destructive insect pest of pulses (MENSAH, 1986). They attack the pods of pulses in the field and are carried to the godown and completely destroy the endosperm of grains causing loss in viability of seeds, unsuitable for human consumption, for sowing or production of sprouts. The huge losses caused by bruchids remains the biggest impediment in the safe storage of pulses. Among different methods, the seed treatment (ST) with botanicals remains cheap, easy to apply and cost effective. The effect of botanicals for their efficacy against the bruchids has not yet been adequately studied under coastal climatic conditions of Odisha (MISHRA *et al*., 2018). Botanicals in general are environmentally safe, less hazardous, less expensive and readily available. Keeping the above aspects in view, the present study was undertaken to study the efficacy of pesticides as botanic origin as seed protectant and effects on infestation and biology of pulse beetle *C. chienensis.*

MATERIALS AND METHODS

Studies were conducted in the laboratory of Seed Technology Research Centre, OUAT, Bhubaneswar to evaluate the performance of some new insecticide molecules against pulse beetle on stored greengram and blackgram seeds**.** The Pulse beetle,

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Callosobruchus chinensis (L.), adults were collected by the help of aspirator and kept in rearing jars which were covered with muslin cloth over the mouth of the jar**.** Matured, well filled and healthy pest-free seeds of greengram (*Vigna radiata* var.Barchana local) and blackgram (*Vigna mungo* var. Barchanalocal) were procured from farmers of Barchana village for experimental purpose**.** The botanicals *viz*., like begunia, naguari, curry leaf powder@10g/kg seed, citronella oil @10 ml and sweet flag formulation 6EC @ 10 ml/kg seed were used as seed protectants. Freshly harvested seeds of greengram and blackgram were dried under sun uptoa moisture content below 10%.The seeds were then fumigated separately in enclosed chamber for 7 days by aluminum phosphide tab @ 1 tab (3 g/cum of space) to destroy the insect infestation. After fumigation entire seed lot 12 kg was aerated so as to remove release of phosphine gas. A total of 12 kg of greengram and blackgram seeds was required for experimental purpose for seed treatments. Laboratory experiment for insecticidal treatments was conducted separately for greengram and blackgram seeds. Required quantity of insecticide (SG/SC/EC formulation) was diluted with 5 ml of water were are mixed thoroughly with 1 kg seed after spreading on polythene sheet. Different polythene sheets were used for different treatment. In control, only 5 ml of water was mixed with the seeds then both greengram or blackgram treated seeds dried separately treatmentwise under fan at room temperature in same polyethene sheet. The treated seeds were filled in 12 files/jars (20 x 15 cm), 6 vials for greengram and 6 vials for blackgram seeds, @ 1 kg/vial and stored. From the stored stalked seeds 200g of greengram and blackgram seeds were removed treatmentwise one day after insecticidal treatment.

Each set of 200 g seeds was divided into 4 parts @ 50g seeds, which formed four replications of each treatment. Each 50g of treated seeds were transferred to a plastic jar (10cm x 7cm). Altogether there were 20 vials for blackgram seeds for initial laboratory experiment. Similarly from stored stalk of insecticidal treated seeds, 200 g of greengram and blackgram seeds were removed treatmentwise after 2, 4 and 6 months of seed treatments conducting 2^{nd}, 3^{rd} and 4^{th} phase of laboratory experiment following same procedure mentioned above. Ten pairs of freshly emerged *C. chinensis* adults were released in each vial filled with treated seeds stored for 24 hours; 2, 4, and 6 months after seed treatment for conducting initial, 1^{st}, 2^{nd} and 3^{rd} phase of laboratory experiment, respectively. Then top of vials were tightly covered with muslin cloth with rubber bands. Observations on per cent mortality of pulse beetles, number of eggs laid/100 seeds, number of adults (F_1 progeny) emerged and seed quality parameters like seed infestation (%) were recorded at different storage periods/ time intervals in storage after seed treatment. Observations on newly emerged of adult beetle (F_1 population) in treated pulse seeds exposed to pulse beetle infestation were made at 2, 4 and 6 months of storage period. The vials were observed daily to note the adult emergence (F_1 progeny) starting 20 days after release. The number of adult beetles (F_1 population) emerged was recorded on each alternate day. The counting was continued till the complete emergence of F_1 progeny. Finally number of adults emerged in each vial content treated seeds was counted. The data obtained on different observations were subjected to statistical analysis after suitable transformation.

RESULTS AND DISCUSSIONS

Adult mortality of *C. chinensis:* Sweet flag formulation showed highest mortality (97.5 %) followed by *citronella* oil (92.5 %) and begunia leaf powder (90.0 5) at 7 days after release (DAR) of pulse beetle in freshly treated greengram seeds (1 DAST). In blackgram it was recorded maximum in sweet flag formulation (100.0 %) followed by begunia leaf powder (95.0 %) and citronella oil (92.5 %). On 7 days exposure of pulse beetle after 6 months of seed treatment sweet flag formulation also registered maximum of 67.5 and 75.0% adult mortality followed by c*itronella* oil (65.0 and 62.5 %) and begunia leaf

powder (62.5 and 65.0 %) in greengram a blackgram seeds, respectively. Naguari leaf powder was least effective and brought about only 45.0% adult mortality after 6 months of seed treatment in both the pulse seeds. Among botanicals sweet flag formulation consistently proved to be most effective followed by alternation in position between *citronella* oil and *Vitex negundo* (begunia*)* leaf powder at different storage intervals in causing the mortality of bruchids. The effect of botanicals was more evidenced in blackgram than greengram seeds against bruchids. Efficacy of sweet flag rhizome powder @ 1-3 % and dried leaf powders of begonia @ 3 % in inducing higher mortality of adult bruchids as grain protectant in pulses has been reported earlier by (MISHRA, 2000). GOVINDON and NELSON (2007) observed cent per cent mortality *C. maculatus* adult in blackgram treated with sweet flag rhizome powder at the rate of 0.75-2.0 % at 48 hours after treatment. *Acorous calamus* 10 D @ 3 % caused 100 % mortality of bruchids after 72 hours and 84 hours of treatment when dust formulations were prepared using chalk and fly ash as filler, respectively. Further, RAHMAN and TALUKDAR (2006) reported that begonia oil extract was more effective of 3 extracts tested *Vitex negundo* (nishinda), *Eucalyptus* and bank 1 mci) and caused highest mortality of *C. maculatus* in treated blackgram seeds. Multi-locational seed entomology trials conducted at ANGRU (Hyderabad), TNAU (Coimbatore), PDKV (Akola), JNU (Jamnagar), and CSAU & T (Kanpur) on various pulse seeds revealed that sweet flag formulation, begunia (nirgudi), *citronella* oil were found promising causing higher mortality of adult bruchids up to 6 months after treatment as compared to others. Thus the present study was in agreement within the results of above scientists.

Oviposition of *C. chinensis:* As per botanicals concerned, all the plant materials proved superior over control as seed protectant in inhibiting the oviposition rate by bruchids. It was noticed that lowest number of eggs were laid in sweet flag formulation (3.75-8.0 eggs) followed by c*itronella* oil treatment (7.0 to 11.50 eggs) during the entire period of study in greengram seeds. Similarly bruchids oviposited lowest number of eggs in blackgram seeds treated with sweet flag formulation (3.25-8.5 eggs) followed by begunia leaf powder (5.75 -10.75 eggs), citronella oil and curry leaf powder showed a maximum of (11.50-12.50 eggs) in greengram and (12.00 & 15.50 eggs) in blackgram after 6 months of seed treatment. Naguari leaf powder was comparatively inferior among botanicals and recorded 29.0 and 26.75 number of eggs at 6 MAST in greengram and blackgram, respectively. RAHMAN and TALUKDAR (2006) stated that powdered leaves of begunia and *Eucalyptus* @ 3% mixture reduced oviposition of beetle in treated blackgram seeds. Decrease fecundity by bruchid infesting pulse seed grains treated with different vegetable oils (edible, non-edible and hair oils) was also reported (BHATANAGAR *et al*., 2001 and SINGH, 2003). These oils had adverse effect on oviposition preference and showed significant repellent, ovipositional deterrent effects for egg-laying by bruchids up to 6 months and resulted in low fecundity in treated seeds. According to PANDEY *et al*. (2008), the contact toxicity of oils was found to be more effective in comparison to fumigant and seed fumigation and exhibited inhibition of oviposition by bruchids on pegionpea seeds. As the *citronella* oil having strong repellent and oviposition deterrent actions, the fecundity was lower, several plant formulations were tested and found promising. In present investigation, sweet flag (TNAU, formulation) showed anti-oviposition effect that reduced the number of eggs on treated seeds.

Population build up (F_1) of *C. chinensis:* Among different botanicals tested minimum number of adults emerged in sweet flag formulation (2.75 to 7.75 adults) followed by citronella oil (5.00 to 11.00 adults) and begonia leaf powder (5.50 to 14.50 adults) from greengram seeds during the period of study. In blackgram, sweet flag formulation also showed minimum population build up (1.75-6.25 adults) followed by begunia (4.75-12.50 adults) and c*itronella* oil (5.75-14.50 adults). These three plant products were more effective in preventing more embryonic development, transformation to F_1 adult bruchids.

Table-1: Residual toxicity of botanicals treated pulse seeds against *Callosobruchus chinensis* L. and the oviposition rate at different time intervals after seed treatment during storage

Treatment & Conc. in ppm (a.i.)	**Mortality (%) of *C. chinensis* at different storage periods (7 DAR)***								**No. of eggs laid/100 seeds***					
	Blackgram				**Greengram**				**Blackgram**			**Greengram**		
	1 DAST	**2 MAST**	**4 MAST**	**6 MAST**	**1 DAST**	**2 MAST**	**4 MAST**	**6 MAST**	**2 MAST**	**4 MAST**	**6 MAST**	**2 MAST**	**4 MAST**	**6 MAST**
Vitex negundo (Begunia leaf powder @10g/kg seed)	95.0 (80.50)	85.0 (67.50)	75.0 (60.12)	65.0 (53.78)	90.0 (71.56)	77.5 (61.78)	67.5 (65.29)	62.5 (52.27)	5.75 (2.50)	7.75 (2.87)	10.75 (3.33)	6.25 (2.60)	10.75 (3.35)	17.00 4.18)
Lantana camara (Naguari leaf powder @10g/kg of seed)	72.5 (58.45)	67.5 (55.28)	55.0 (47.89)	45.0 (42.12)	65.0 (53.80)	57.5 (49.33)	47.5 (43.56)	45.0 (42.11)	9.00 (3.08)	18.50 (4.35)	26.75 (5.21)	9.75 (3.20)	14.25 (3.83)	29.00 (5.42)
Murraya koenegii (Curry leaf powder @10g/kg of seed)	85.0 (67.50)	77.5 (61.78)	62.5 (52.28)	57.5 (49.33)	77.5 (61.78)	65.0 (53.78)	55.0 (47.89)	55.0 (47.89)	8.25 (2.95)	9.25 (3.11)	15.50 (4.00)	8.25 (2.95)	13.0 (3.67)	12.50 (3.60)
Citronella mucronata (*Citronella* oil @10 ml/kg of seed)	92.5 (76.30)	82.5 (65.47)	72.5 (58.45)	62.5 (52.28)	92.5 (76.02)	72.5 (58.40)	72.5 (58.40)	65.0 (53.78)	7.75 (1.91)	11.0 (3.38)	12.0 (3.53)	7.00 (2.73)	9.75 (3.19)	11.50 (3.45)
Acorous calamus (sweet flag formulation @10 ml/kg of seed)	100.0 (89.43)	90.0 (71.56)	77.5 (61.78)	75.0 (60.12)	97.5 (84.96)	85.0 (67.50)	77.5 (61.77)	67.5 (55.29)	3.25 (1.91)	5.00 (2.34)	8.50 (2.99)	3.75 (2.06)	5.75 (2.49)	8.00 (2.91)
Untreated control	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)	18.50 (4.35)	61.50 (7.87)	82.50 (9.11)	25.50 (5.08)	65.25 (8.10)	90.50 (9.53)
SEM(±)	3.02	1.56	1.54	1.58	2.76	1.64	1.45	1.48	0.11	0.10	0.12	0.09	0.12	0.13
CD(0.05)	8.98	4.64	4.58	4.51	8.20	4.88	4.31	4.39	0.33	0.30	0.35	0.26	0.35	0.38

* Mean of 4 replications, DAR = Days after release, DAST = Days after seed treatment, MAST = months after seed treatment; Figures in parentheses are transformed values

Table-2: Effect of botanicals on population build up (F_1 progeny) of *Callosobruchus chinensis* (L.) in treated pulse seeds at bi-monthly intervals during storage and seed damage in treated pulse seeds at 2 months storage intervals after seed treatment

Treatment	No. of adults (F_1 progeny)						Seed damage (%)					
	Blackgram			Greengram			Blackgram			Greengram		
	2 MAST	4 MAST	6 MAST	2 MAST	4 MAST	6 MAST	2 MAST	4 MAST	6 MAST	2 MAST	4 MAST	6 MAST
Vitex negundo (Begunia leaf powder @10g/kg seed)	4.75 (2.27)	7.00 (2.72)	12.50 (3.59)	5.50 (2.44)	8.50 (2.99)	14.50 (3.85)	0.75 (1.09)	1.25 (1.31)	1.50 (1.40)	1.50 (1.40)	2.25 (1.63)	2.50 (1.70)
Lantana camara (Naguari leaf powder @10g/kg of seed)	10.25 (3.26)	20.50 (4.56)	25.75 (5.10)	12.50 (3.60)	23.00 (4.84)	30.00 (5.48)	1.75 (1.49)	3.25 (1.93)	3.75 (2.06)	2.25 (1.65)	3.75 (2.05)	5.25 (2.39)
Murraya koenegii (Curry leaf powder @ 10g/kg of seed)	7.75 (2.87)	15.25 (3.95)	19.25 (4.42)	9.75 (3.19)	15.25 (3.97)	18.50 (4.34)	1.50 (1.40)	1.75 (1.49)	3.00 (1.86)	2.00 (1.56)	2.50 (1.70)	4.25 (2.17)
Citronella mucronata (*Citronella* oil @10 ml/kg of seed)	5.75 (2.49)	8.25 (2.94)	14.50 (3.85)	5.00 (2.33)	9.50 (3.16)	11.0 3.37)	1.00 (1.22)	1.50 (1.40)	2.25 (1.65)	1.25 (1.31)	2.25 (1.65)	2.50 (1.72)
Acorous calamus (sweet flag formulation @10 ml/kg of seed)	1.75 (1.49)	3.75 (2.04)	6.25 (2.56)	2.75 (1.79)	6.75 (2.68)	7.25 (2.76)	0.50 (0.96)	1.25 (1.31)	1.75 (1.49)	1.25 (1.31)	1.75 (1.49)	2.25 (1.63)
Untreated control	22.50 (4.78)	66.25 (8.16)	80.75 (9.01)	30.75 (5.55)	73.0 (8.58)	85.25 (9.28)	4.25 (2.17)	8.50 (2.99)	11.50 (3.46)	4.50 (2.23)	10.75 (3.35)	13.00 (3.66)
SEM (±)	0.15	0.17	0.23	0.19	0.11	0.22	0.09	0.09	0.09	0.09	0.12	0.13
CD (0.05)	0.44	0.51	0.69	0.55	0.32	0.66	0.26	0.26	0.26	0.26	0.36	0.39

*Mean of 4 replications, MAST – Month after seed treatment; Figures in parentheses are transformed values

Naguari leaf powder was comparatively less effective and harboured a maximum of 30.00 and 25.75 adults as compared to 85.25 and 80.75 adults in untreated control after 6 months of treatment in greengram and blackgram seeds, respectively. Efficacy of different plant materials as seed protectant (turmeric rhizome powder, neem leaf powder, black pepper seed powder and sclore powder etc.) in reducing the emergence of adult bruchids (F_1 population) on pulse seeds up to 4 months of storage had been studied previously by GILL and SINGH (2012), NEOG and SINGH (2013). In present endeavor the c*itronella* oil suppressed the emergence of adult beetle (F_1 population) mainly contributed due to its characteristic odour and fumigant and contact toxicity against eggs.

Seed damage by *C. chinensis:* All the botanicals (leaf powder, plant oil and formulations) had profound influence in minimizing seed infestation by bruchids to a great extent. Among botanicals, sweet flag formulation controlled the pest most effectively and showed least infestation (1.25-2.25 %) in treated greengram seeds followed by c*itronella* oil (1.25-2.50 %) and begonia leaf powder (1.50-2.50 %) through entire period of investigation. Seed treatment with curry and naguari leaf powder contributed moderate seed damage of 4.25-5.25%, respectively as compared to 13.00 % in control. Botanical treatments in greengram seeds contributed reduction of 59.61 % to 82.69 % over control after 6 months of storage period. In blackgram, begunia leaf powder gave maximum protection to treated seeds (1.50 %) followed by sweet flag formulation 1.75 % and citronella oil (2.25 %) after 6 months of storage. A reduction advantage of 67.39 to 86.96 % in seed damage over control was estimated in different botanical treatments after 6 months of storage in blackgram seeds. All the botanicals provided better protection of blackgram seeds against bruchids than that of greengram. RAHMAN and TALUKDAR (2006) viewed that powdered leaves and extracts of nisinda (begonia), *Eucalyptus* and bankalmi at a 3 % mixture decreased the seed infestation considerably and provided excellent protection against *C*. *maculatus* in blackgram. Pulse seeds smeared with different types of plant oils @ 8 to 15 ml/kg of seeds minimized the seed infestation up to 4-9 months storage period were reported by KHANNA (1995), SINGH, 2003 and JANGAMASHETTI *et al*. (2008). Multilocational seed entomology trials conducted at different centers that is TNAU (Coimbatore), CSAUST (Kanpur), PDKV (Akola), JAU (Jamnagar), UAS (Bangalore) and NDUAT (Faizabad) revealed least seed infestation by bruchids up to 6 months storage period when the pulse seeds were treated with sweet flag formulation, begonia leaf powder and citronella oil than other botanicals (Annual Report, AICRP on STR, 2014-15).

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REFERENCES

BHATANAGAR, A., BHADAURIA, N. S. ABD JAKHMOLA, S. S. 2001. Efficacy of vegetable oils against pulse beetle, *Callosobruchus maculatus* in cowpea. *Indian J. Insect Sci.* **63**(3): 237-239.

GILL, R.S. and SINGH, D. 2012. Efficacy of some plant powders as grain protectants against *Callosobruchus chinensis* (Linnaeus) on chickpea. *J. Insect Sci.* **25**(2): 214-219.

GOVINDAN, K. and NELSON, J. S.2007. Effect *Acorus calamus* rhizome powder and Its dust formulations on Oviposition, adult emergence and seed damage on *Callosobruchus maculatus* (F.). *J. Plant Protec. Environ.***4**(1): 70-74.

JANGAMASHETTI, V., SINGAL, S.K. and BHANOT, J.P.2008.Efficacy of various grain protectants against pulse beetle, *Callosobruchus chinensis* (L.) infesting pegionpea, *Cajanus cajan* (L.) Millsp. *J. Insect Sci.* **21**(4): 348-351.

KHANNA S.C. 1995. Efficacy of black pepper in combination with mustard oil in protecting greengram against by *C. chinensis and C. analis* (F.). *Annals of Plant Protec. Sci*., **3** (2): 164-190.

MENSAH, G. W. K. 1986. Infestation potential of bruchids on cowpea cultivars stored under subtropical conditions. *Fac. Agril. Univ. Swaziland* **7**(6): 781-784

MISHRA NAMASHYA, D. DASH, S. K. MOHANTY, B. BAISAKH and P. R. MISHRA, 2018. Efficacy of new molecules of insecticides as seed protectants of greengram and blackgram against pulse beetle, *Callosobruchus chinensis* Linn. *J. Appl. Zool. Res*. **29**(1): 78-84.

NEOG, P. and SINGH, H. K. 2013. Efficacy of some indigenous plant powders against *Callosobruchus chinensis* (L.) infesting rice bean. *Indian J. Ent.* **75**(3*):*203-207.

PANDEY, A.K., MISHRA, A.K., SINGH, P. and TRIPATHI, N. N. 2011. Effect of some essential oils on the oviposition and emergence of *Callosobruchus* spp*. Indian J. Ent.* **73**(1): 10-14.

RAHMAN, A. and TALUKDAR, F. A. 2006. Bioefficacy of some plant derivetives that protect grain against the pulse beetle, *Callosobruchus maculatus*. *J. Insect Sci*.**6** (1):1-10.

SINGH, P.2003. Effect of some oils against pulse beetle, *Callosobruchus chinensis* in infesting pigeonpea.*Indian J. Ent.* **65**(1): 55-58.

SINGH, S. C. and PANDEY, N. K. 1995. Effect of neem on the mortality of the pulse beetle (*Callosobruchus chinensis* L.) on blackgram. *J. Ecotoxicology and Environ. Monitoring*, **5**(3-4): 217-218.

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BIOCHEMICAL ALTERATION IN RICE INFECTED WITH SHEATH BLIGHT DISEASE CAUSED BY *RHIZOCTONIA SOLANI* KUHN

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ABSTRACT: Observations were recorded on various biochemical parameters like soluble protein, soluble sugar and total free amino acids content in healthy and inoculated leaf sheath tissues of a sheath blight tolerant rice variety 'Pankaj' and susceptible variety 'Tapaswini' inoculated with *Rhizoctonia solani* Kuhn. There was decrease in soluble protein, soluble sugar and total free amino acids content during post period infection in the inoculated leaf sheath tissues as compared to that of healthy leaf sheaths. But, on the other hand, soluble protein and soluble sugar content in the healthy and inoculated leaf sheath tissues were found to be more in tolerant variety 'Pankaj' than in susceptible variety 'Tapaswini', whereas total free amino acids content in healthy and inoculated leaf sheath tissues of the susceptible variety 'Tapaswini' was observed to be more than in the tolerant variety 'Pankaj'.

Key words: Rice, *Rhizoctonia solani*, susceptible, tolerant, biochemical changes

INTRODUCTION

Sheath blight caused by *Rhizoctonia solani* Kuhn, is one of the potentially serious diseases of rice next to blast in importance (MANIBHUSANRAO, 1995). The disease was first reported in Japan (MIYAKE, 1910) and became a serious problem after the introduction of high yielding N-responsive cultivars exhibiting grayish green oblong patches on leaf sheaths with reddish brown border, sometimes overlapping and spreading to the leaves and in severe cases, stunting of plants resulting in yield reduction. A modest estimation of losses due to sheath blight disease alone in India has been reported up to 54.3% (RAJAN, 1987; ROY, 1993). Besides fungicidal chemicals, bio-control agents are considered as one of the effective and eco-friendly means of management of this disease affecting rice crop. Several fungi like *Trichoderma viride, T. harzianum, T. koningii* (ROY and SAYRE, 1984; BHAGAWATI, 1994; DUBEY, 1998; SUDHAKAR *et al.*, 1998; *DAS* and HAJZARIKA, 2000;), *Aspergillus niger* (GOKULAPALAN and NAIR, 1984; SEN *et al.,* 1993; KANDHARI *et al.,* 2000), *A. terreus* (DAS, 1992; GOGOI and ROY, 1993), *Gliocladium virens* (BABY and MANIBHUSANRAO, 1993) of rice field are found to be antagonistic against *R. solani*. Antagonistic effect of fluorescent and non-fluorescent *Pseudomonas fluorescens* isolated from rice rhizosphere of Southern India was tested against *R.solani* which inhibited mycelial growth, affected sclerotial viability *in vivo* and protected IR 20 and TKM-9 rice seedlings from infection by *R. solani* in greenhouse tests. Pretreatment of sclerotia in bacterial suspensions resulted in reduction in lesion size up to 31-44% in IR 20 and 58-74% in rice cv. TKM 9. In field plots, IR 20 and TKM 9 rice plants raised from bacteria treated seeds exhibited 65-72% less sheath blight incidence than those plants from untreated seeds (DEVI *et al.*, 1989). The host parasite interaction in essence, is a struggle for survival for both. This brings physiological and biochemical changes in the host tissues, which are usually reflected by visual symptoms. In the present investigation, attempts have been made to study the biochemical changes with special reference to

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changes in soluble protein, soluble sugar and total free amino acids in the host tissues, brought about by establishment of the fungus causing sheath blight disease after affecting normal physiology of rice plants.

MATERIALS AND METHODS

Seeds of susceptible variety 'Tapaswini' and tolerant variety 'Pankaj' were grown in hot water disinfected earthen pots (16 cm diameter and 20 cm depth) filled with steam sterilized manure enriched soil (soil 3 parts, manure 1 part). Two seedlings were transplanted in each pot followed by regular watering to maintain adequate level of moisture. Fifteen isolates of *Rhizoctonia solani* Kuhn were inoculated to the rice plants of above two varieties grown individually in earthen pots at maximum tillering stage, where two potted plants were inoculated with each isolate for 'Pankaj' and 'Tapaswini' varieties and two sets of plants were left uninoculated to serve as control. Samples (leaf sheaths) were collected twenty days after inoculation.

Estimation of soluble protein:

Soluble protein was estimated by the method described by LOWRY *et al.* (1951). Soluble protein was extracted in 0.1 Molar cold Sodium phosphate buffer. About 0.5 gram of the sample i.e. leaf sheath from potted rice plants of 'Pankaj' and 'Tapaswini' varieties (inoculated with 15 isolates of *R. solani* and healthy ones i.e. without inoculation with any of the isolates) were collected, chopped into pieces with scissors and ground well with a pastle and mortar in 5 ml of the buffer. The extract was cold centrifuged at 14000 rpm for 20 minutes. The supernatant was decanted and used for protein estimation. In a test tube 0.5 ml of the extract was taken. The volume was made up to 1 ml by adding with double distilled water. Five milliliter of alkaline copper solution was added to the tube including the blank. It was mixed properly and allowed to stand for 10 minutes. Then 0.5 ml of 1 N Folin's reagent was added, mixed well and inoculated at room temperature in the dark for 30 minutes. The absorbance was measured at 660 nm by the help of a Spectrophotometer. Soluble protein content was expressed as mg protein/ fresh weight of sample.

Estimation of soluble sugar:

Soluble sugar was estimated by the method described by YOSHIDA *et al.* (1976). About 0.5 g samples i.e. leaf sheaths from potted rice plants of 'Pankaj' and 'Tapaswini' varieties (inoculated with 15 isolates of *R. solani* and healthy ones i.e. without inoculation with any of the isolates) were collected, chopped into 15 ml centrifuge tubes. Ten milliliter of 80% ethanol was added to each of the tubes. A glass ball was placed on the top of the tube and kept in a hot water bath at 80-85^{o} C for 30 minutes. Then after cooling, it was centrifuged and the supernatant was decanted into a 50 ml beaker. This extraction was repeated thrice. The alcohol extract was evaporated on a water bath until most of the alcohol was removed (*e.g.* the volume was reduced to about 2 ml). The sap was transferred into a 50 ml volumetric flask after rinsing thoroughly with distilled water 4-5 times and the volume was made up to 50 ml.

Two milliliters of sugar extract was transferred in to a 50 ml volumetric flask and volume was made up to 50 ml by adding with distilled water. Five milliliter of this extract was taken in a 25 ml volumetric flask. Simultaneously standards of 0.1 ml, 1.5 ml and 2 ml of 100ppm glucose solution were taken in 25 ml volumetric flask. Volume of these standards was made up to 5 ml with addition of distilled water and 2 drops of 80% ethanol. Volumetric flask containing samples and standards were kept in an ice-bath. To the volumetric flask, 10 ml of anthrone reagent (2 grams of anthrone in 1 litre of 95% H_2SO_4) was added allowing it to run down the side of the volumetric flask. The contents of the flasks were shaken slowly by swirling the flasks and then shaken thoroughly. The

volumetric flasks were kept in boiling water bath exactly for 7.5 minutes. Then immediately the flasks were cooled in ice. After cooling, absorbance was measured at 630nm by the help of a spectrophotometer and the sugar content (in mg/g fresh weight of the sample) was calculated from the standard curve.

Estimation of total amino acid

Total free amino acids were estimated by SADASIVAM and MANICKAM (1992). Approximately, 0.5g of sample (leaf sheath) collected as in the above similar way was weighed, chopped finely and ground in a pastle and mortar with a small quantity of acid washed sand. To this homogenate, 10 ml of 80% ethanol was added till the samples were macerated to pulp. These materials after grinding were boiled for 5-10 minutes in a hot water bath. After boiling, the alcoholic extracts were filtered in a double layered fine cheese cloth so as to remove the floating plant debris. The extracts were centrifuged at 5000rpm for 10 minutes. All the suspended materials were settled at the bottom and top portion became very clean. The supernatant was reduced by keeping on a hot water bath. This extract was used for amino acid estimation.

One milliliter of ninhydrine solution was added to 0.1 ml of extract. The volume was made up to 2 ml with distilled water. The tube was heated in a boiling water bath for 20 minutes. Five milliliter of diluent was added and the contents were mixed properly. After 15 minutes the intensity of the purple colour was read aginst a reagent blank in a Colorimeter at 570 nm. The reagent blank was prepared as above by taking 0.1 ml of 80% ethanol instead of the extract. Fifty milligrams of leucine was dissolved in 50 ml of distilled water in a volumetric flask. Ten milliliters of this standard was taken and diluted to 100 ml another volumetric flask for working standard solution. A series of volume from 0.1 to 1 ml of this standard solution gave a concentration range from 10 µg to 100 µg. A standard curve was drawn from the absorbances of the known standard solutions. The concentration of the total free amino acid in the sample was calculated from the standard curve.

RESULTS AND DISCUSSION

Data presented in Table 1 revealed that there was decrease in soluble protein content in the inoculated leaf sheath tissues of both susceptible cultivar ‘Tapaswin’ and tolerant cultivar ‘Pankaj’ inoculated by different isolates of *R. solani*. It was also found that the protein content was more in tolerant variety ‘Pankaj’ than in susceptible variety ‘Tapaswini’ even after inoculation with corresponding isolates. Soluble protein content in both the susceptible and tolerant varieties was observed to be minimum when inoculated with S_2 isolate of *R. solani.* The present study relating to interaction of rice host plant and sheath blight pathogen revealed a reduction in soluble protein content of the inoculated host plants with that of the healthy ones both in susceptible variety ‘Tapaswini’ and tolerant variety ‘Pankaj’ where the soluble protein content was found to be relatively higher in the later than the former. Contrary to the earlier findings of MEHROTRA (1980), the decrease in soluble protein content might be due to continuous utilization of protein sources by the pathogen and the host to meet energy requirement and withstand the infection caused by the pathogen. The post-infectional decrease in protein level appeared to be associated with the susceptibility of the host as suggested by GANGULY (1995).

Table-1: Changes in soluble protein in healthy and inoculated rice plant parts (leaf sheath tissues) inoculated with *Rhizoctonia solani* Kuhn

Sl. No.	Isolate (s)	Soluble protein in mg/g fresh		Mean
		Var. Tapaswini	Var. Pankaj	
1	S_1	2.191	3.676	2.933
2	S_2	1.939	2.490	2.215
3	S_3	2.811	3.130	2.970
4	S_4	3.543	3.987	3.765
5	S_5	2.967	3.337	3.152
6	S_6	2.019	3.906	2.963
7	S_7	3.113	4.473	3.793
8	S_8	2.067	2.630	2.349
9	S_9	2.091	2.602	2.346
10	S_{10}	2.108	3.079	2.594
11	S_{11}	2.086	3.005	2.546
12	S_{12}	2.511	3.107	2.809
13	S_{13}	2.966	3.364	3.165
14	S_{14}	3.335	4.525	3.930
15	S_{15}	2.708	2.867	2.788
16	Healthy	3.779	4.967	4.373
	Mean	2.640	3.447	
CD (0.05)	Variety (0.272) , Isolates (0.470)			
±SE	Variety (0.014), Isolates (0.041)			
CV%	2.72			

Estimation of soluble sugar

The experimental findings presented in Table-2 indicated that there was reduction in soluble sugar content in both the susceptible and tolerant cultivars when inoculated with the individual isolates of *R. solani* in comparasion to healthy leaf sheath tissues. Invariably the sugar content of the inoculated tolerant variety 'Pankaj'was found to be more than the susceptible variety 'Tapaswini' irrespective of the isolates. The reduction in sugar content in comparison to the healthy ones were found to be statistically at par in the isolate S_5 and S_8, whereas, the rest of the isolates recorded a significant difference among the isolates with that of the healthy ones. The soluble sugar content recorded in the susceptible variety 'Tapaswini' and the tolerant variety 'Pankaj' by the isolate S_4 and S_{12} were found to be statistically at par with that of the healthy ones, but the rest of the isolates behaved differently in utilizing the soluble sugar sources of the host tissues. This is in agreement with the earlier findings of RAMLINGAM (1982), ZUBER and MANIMHUSANRAO (1983).

Estimation of total free amino acids

The experimental findings presented in Table-3 revealed that there was reduction in total free amino acids in the leaf sheath tissues of both the susceptible cultivar 'Tapaswini" and tolerant cultivar 'Pankaj' when inoculated with the individual isolates of R.solani in comparision to healthy leaf sheath tissues. When both the susceptible and tolerant

varieties were inoculated with the test isolates of *Rhizoctonia solani* individually, reduction in total free amino acids content could be in both the cases showing a significant difference among the isolates except in S_4 and S_8 isolates, S_{12} and S_{13} isolates, S_{13} and S_{14} isolates found to be

Table-2: Changes in soluble sugar in healthy and inoculated rice plant parts (leaf sheath tissues) inoculated with *Rhizoctonia solani* Kuhn

Sl. No.	Isolate (s)	Soluble protein in mg/g fresh		Mean
		Var. Tapaswini	Var. Pankaj	
1	S_1	3.858	4.476	4.167
2	S_2	4.334	4.430	4.370
3	S_3	3.111	3.874	3.493
4	S_4	3.316	4.207	3.762
5	S_5	4.590	4.818	4.704
6	S_6	3.844	4.622	4.233
7	S_7	3.355	4.196	3.775
8	S_8	4.607	5.269	4.938
9	S_9	4.560	5.432	4.996
10	S_{10}	4.124	5.051	4.588
11	S_{11}	3.952	4.259	4.106
12	S_{12}	3.966	4.074	4.020
13	S_{13}	3.043	4.889	3.966
14	S_{14}	2.357	4.056	3.206
15	S_{15}	2.642	4.575	3.609
16	Healthy	5.254	6.325	5.789
	Mean	3.806	4.660	
CD (0.05)	Variety (0.242), Isolates (0.689)			
±SE	Variety (0.084), Isolates (0.239)			
CV%	11.33			

Table- 3: Changes in total free amino acids in healthy and inoculated rice plant parts (leaf sheath tissues) inoculated with *Rhizoctonia solani* Kuhn

Sl. No.	Isolate (s)	Soluble protein in mg/g fresh		Mean
		Var. Tapaswini	Var. Pankaj	
1	S_1	0.609	0.315	0.462
2	S_2	0.252	0.222	0.237
3	S_3	0.837	0.298	0.568
4	S_4	0.434	0.220	0.327
5	S_5	0.373	0.285	0.329
6	S_6	0.541	0.205	0.373
7	S_7	0.632	0.221	0.427
8	S_8	0.713	0.222	0.468

Contd…Table-3

Table-3. Contd…

Sl. No.	Isolate (s)	Soluble protein in mg/g fresh		Mean
		Var. Tapaswini	Var. Pankaj	
9	S_9	0.548	0.372	0.460
10	S_{10}	0.600	0.437	0.519
11	S_{11}	0.703	0.317	0.510
12	S_{12}	0.466	0.227	0.347
13	S_{13}	0.438	0.220	0.329
14	S_{14}	0.487	0.476	0.482
15	S_{15}	0.397	0.200	0.298
16	Healthy	0.881	0.567	0.724
	Mean	0.557	0.300	
CD (0.05)	Variety (0.020), Isolates (0.063)			
±SE	Variety (0.007), Isolates (0.222)			
CV%	10.46			

statistically at par in susceptible variety 'Tapaswini' and the isolates S_2, S_4, S_5, S_7, S_6, S_{12}, S_{13} and S_{15} in tolerant variety 'Pankaj'. However, the lowest content of total free amino acids (0.252 mgt/g fresh weight) was recorded in the variety 'Tapaswini' inoculated with S_2 isolate and 0.200 mg/g fresh weight in the variety 'Pankaj' inoculated with S_{15} isolate followed by 0.205 mg/g fresh weight inoculated with S_6 isolate of *R. solani*. Extraction of highest total free amino acids was made in the variety 'Tapaswini' inoculated with S_3 (0.837 mg/g fresh weight) isolate followed by S_8 (0.713 mg/g fresh weight) isolate and S_{11} (0.703 mg/g fresh weight) isolate of *R. solani*, whereas in tolerant variety 'Pankaj' highest quantity of total free amino acids could be obtained by inoculation with S_{14} isolate (0.476 mg/g fresh weight) followed by S_{10} isolate (0.437 mg/g fresh weight). This is in conformity with earlier findings of ZUBER and MANIBHUSANRAO (1983), YOSHIDA (1981).

REFERENCES

BABY, V. I. and MANIBHUSANRAO, K. 1993. Control of rice sheath blight through the integration of fungal antagonists and organic amendment. *Tropical Agric*., **70**: 240-244

BHAGAWATI, R. 1994. Effect of ecological factor and the survival of *Trichoderma* spp. in relation to suppressing the incidence of sheath blight of rice. *Ph. D. Thesis, AAU, Jorhat, Assam, pp* 143.

DAS, B.C. and HAZARIKA, D.K. 2000. Biological management of sheath blight of rice. *Indian Phytopath.,* **53**: 433-435

DAS, D.C. 1992. Antagonism of *Aspergillus terreus* on *Rhizoctonia solani* and its effect on the incidence of sheath blight of rice. *Ph.D Thesis, AAU, Jorhat, Assam,* pp175*.*

DEVI, T.V., VIZHI, R.M., SAKTHIVEL, N. and GNANAMANICKAM, S.S. 1989. Biological control of sheath blight of rice in India with antagonistic bacteria. *Plant and soil* **119**(2): 325-330.

DUBEY, S.C. 1998. Evaluation of different fungal antagonists, plant extracts and oil cakes against *Thanatephorus cucumeris* causing banded blight of rice. *J. Mycol. Pl. Pathol.,* **28**: 266-269.

GANGULY, L.K. 1995. Biochemical alterations in respect of total phenol, sugar and protein contents in *R. solani* Kuhn inoculated rice plants. *J. Mycopathol. Res.* **33** (1): 53-58.

GOGOI, R. and ROY, A.K. 1993. Effect of foliar spray of *Aspergillus terreus* on sheath blight of rice. *Int. Rice Res. Notes,* **18**: 31-32

GOKULAPALAN, C. and NAIR, M.C. 1984. Antagonism of a few fungi and bacteria against *Rhizoctonia solani* Kuhn. *Indian J. Microbiol.*, **24**: 57-58

KANDHARI, J., MAJUMDAR, S. and SEN. B. 2000. Impact of *Aspergillus niger* AN27 for growth promotion and sheath blight reduction in rice. *Int. Rice Res. Notes,* **25**: 21-22.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. and RANDALL, R.J. 1951. Protein measurement with the folin-phenol reagent. *J.Biology and Chemistry* **193**: 265-290.

MANIBHUSANRAO, K. 1995. Sheath blight disease of rice. *Daya Publishing House, New Delhi, India,* pp 101.

MEHROTRA, R.S. 1980. Plant Pathology. *Tata- McGraw Hill Publishing Company Limited., New Delhi,* pp 771.

MIYAKE, I. 1910. Studien uber die Pilze der Reispflanze in Japan. *J. College of Agriculture, Imperial University of Tokyo,* **2**: 237-276.

RAJAN, C.P.D. 1987. Estimation of yield losses due to sheath blight disease of rice. *Indian Phytopath*. **49**(2) : 174-177.

RAMLINGAM, P. 1982. Changes in amino acid content of healthy and *Rhizoctonia solani* infected rice tissues. *Current Science* **51**: 278-279.

Roy,A.K. and Sayre, R.M. (1984). Electron microscopy studies of *Trichoderma harzianum* and *T. viride* and mycoparasitic activity of the former on *Rhizoctonia solani* f.sp. *sasakii*. *Indian Phytopath*., **37**: 710-712

ROY, A.K. 1993. Sheath blight of rice. *Indian Path*. **46**: 197-205

ROY, A. K. and SAYRE, R. M.1984. Electron microscopy studies of *Trichoderma harzianum* and *T. viride* and mycoparasitic activity of the former on *Rhizoctonia solani* f.sp. *sasakii*. *Indian Phytopath*., **37**: 710-712.

SADASIVAM, S. and MANICKAM, A. 1992*. Biochemical methods for Agricultural Sciences Wiley Eastern Limited, New Delhi*, pp 40-42.

SEN, B., SARMA, J., ASALMOL. M. N. C., CHATTOPADHYA, C. and PATIBANDA, A. K. 1993. *Aspergillus niger*- a potential bio-control agent for soil borne pathogen. *Indian Phytopath*., **46**: 275.

SUDHAKAR, R., RAO, K. C. and REDDY, C. S. 1998. Studies on antagonism of *Trichoderma* and *Gliocladium* species against *R. solani* of rice. *Indian J. Plant Prot.*, **26**: 25-29.

YOSHIDA, S. F., DOUGHLAS, A., COOK, J. A. and GOMEZ, K. A. 1976. *Laboratory Manual for physiological studies of rice. IRRI, Los Banos, Phillipines*, pp 46-47.

YOSHIDA, S. 1981. Fundamentals of rice crop science. *Int. Rice Res. Inst.*, *Los Banos, Philippines,* pp 269.

ZUBER, M. and MANIBHUSANRAO, K**.** 1983. Physiology of host pathogen interaction in rice sheath blight with reference to carbohydrate & nitrogen contents. *Indian J. Bot*., **6**: 122-124.

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APPROXIMATE DIGESTIBILITY (A.D.) AND E.C.D. OF THE LEMON BUTTERFLY, *PAPILIO DEMOLEUS* (LINN.) STUDIED UNDER LABORATORY CONDITIONS AT RAIPUR

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ABSTRACT: Lemon is attacked by a number of insect pests and among them lemon butterfly, *Papilio demoleus* (Linn.) is the most common defoliator pest. The present study on food consumption and utilization by the lemon butterfly, *Papilio demoleus* (L.) was conducted at different instars in the Bio-control Laboratory, Department of Entomology, IGKVV, Raipur, Chhattisgarh, during May, 2018. Results indicated that the amount of digested food (A.D.) was highest for 2nd instar larva (85.68%) and lowest for 6th instar larva (23.33%). The conversion of food into tissue (E.C.D.) was highest for 5th instar larva (84.63%) and lowest in 2nd instar larva (13.55%) which indicated highest food utilization in 2nd larval instar and highest conversion into tissue in the 5th instar.

Key words: Approximate digestibility, E.C.D., citrus lemon butterfly, *Papilio demoleus*

INTRODUCTION

The Lemon, *Citrus lemon* (Linn.) is a species of small evergreen tree in the flowering plant of family Rutaceae, native to South Asia. Citrus fruits are notable for their fragrance, partly due to flavonoids and limonoids contained in the rind, and most are juice-laden. The juice contains a high quantity of citric acid giving them their characteristic sharp flavor (SATYAGOPAl *et al*., 2014). Insect pests of citrus include citrus psylla (*Diaphorina citri*), citrus whitefly (*Dialeurodes citri*), citrus blackfly (*Aleurocanthus woglumi),* cottony cushion scale (*Icerya purchasi*), citrus mealy bug (*Psuedococcus filamentosus*), citrus lemon butterfly (*Papilio demoleus*), citrus leafminer (*Phyllocnistis citrella),* fruit sucking moth (*Ophiderus conjucta*), citrus aphid (*Toxoptera citricidus*), and citrus mite (*Oligonichus citri*).

Citrus lemon butterfly, *Papilio demoleus* is a common and widespread swallowtail found from Asia to Australia. Omnipresence of *P. demoleus* indicates the butterfly tolerance and adaptation to diverse habitats. In India, it is mostly found in the plains but can be found on the hills of peninsular India and upto 7,000 feet (2,100m) in the Himalayas. This butterfly is an avid mud-puddler and visitor of flowers. The lemon butterfly is one of the economically important pests whose larval forms cause serious damage by devouring large quantity of foliage of Rutaceae family with special preference towards both wild and cultivated species of citrus during the later stages of their development **(**SARADA and GOPAL, 2013**).** We studied instar wise feeding efficiency of the test insect as one of the physiological parameters of the digestive system and is of academic importance.

MATERIAL AND METHODS

Eggs of lemon butterfly were collected from the lemon orchard at horticultural field, IGKV Raipur, Chhattisgarh during 2018. Collected eggs were used for the experimental purpose. The experiment was conducted at room temperature under laboratory condition during May 2018. Collected eggs were kept in petriplates in the Bio-control Laboratory at

room temperature. The eggs hatched in 2 days (incubation period 3-4 days in summer). Twenty neonate larvae were taken and weighed. Then fresh tender leaves of lemon was weighed and given to the larvae for feeding in petriplate (SANDHU and SANDHU, 2011). After 24 hours weight of the larva, weight of the excreta and weight of the leaves provided were noted. The procedure was repeated after every 24 hours until the larvae reached to pupal stage. The change in larval instars was confirmed by the shed head capsule. The experiment was conducted for the calculation of A.D. and E.C.D. for different instars of larvae of lemon butterfly.

Growth and development indices (physiological parameters) such as Approximate Digestibility (A.D.) and Efficiency of Conversion of Digested Food (E.C.D.) into tissue were calculated at larval instar of lemon butterfly by the formula given by WALDBAUER (1968) as mentioned below:

$$\text{A.D.} = \frac{\text{Amount of food ingested} - \text{Amount of faeces}}{\text{Amount of food ingested}} \times 100$$

$$\text{E.C.D.} = \frac{\text{Weight gained by the insect at a given time}}{\text{Amount of food ingested} - \text{Amount of faeces}} \times 100$$

RESULTS AND DISCUSSION

As per the data in Table, the approximate digestibility (A.D.) was found highest for 2nd instar and 1st instar larva of 85.68 and 79.78 per cent, respectively and lowest in 6th instar larva (23.33%). This is due the fact that the weight of excreta was found to be highest in 6th instar larva (12.11gm) and lowest for 2nd instar larva (0.45gm). The present study is in agreement with DAVEY, 1954 who also stated higher A.D. value in first instar larvae of *Schistocerca.* The ECD values in the present studies indicated maximum in 5th instar (84.63%) which showed that the 5th instar larva utilized the maximum ingested food maximum for conversion into tissue, which is higher than the value given by WALDBAUER, 1968.

Table: Showing amount of food consumption and excretion at different larval instars of the lemon butterfly, *P. demoleus* (L.).

S. No.	Larval Instars	Wt. of Larvae (gm)	Wt. of given leaves (gm)	Wt. of leaves remaining after feeding (gm)	Amt. of food ingested (gm)	Wt. of excreta (gm)	A.D (%)	E.C.D (%)
1.	1st instar larva	0.111	4.00	2.565	1.434	0.23	79.78	9.69
2.	2nd instar larva	0.472	6.00	2.856	3.144	0.45	**85.68**	13.55
3.	3rd instar larva	3.286	12.00	4.683	7.297	2.09	71.35	54.04
4.	4th instar larva	4.504	10.00	0.482	9.518	3.32	62.98	20.31
5.	5th instar larva	7.175	12.00	3.24	8.786	5.63	35.92	**84.63**
6.	6th instar larva	9.12	24.00	8.20	15.806	12.11	23.33	52.79

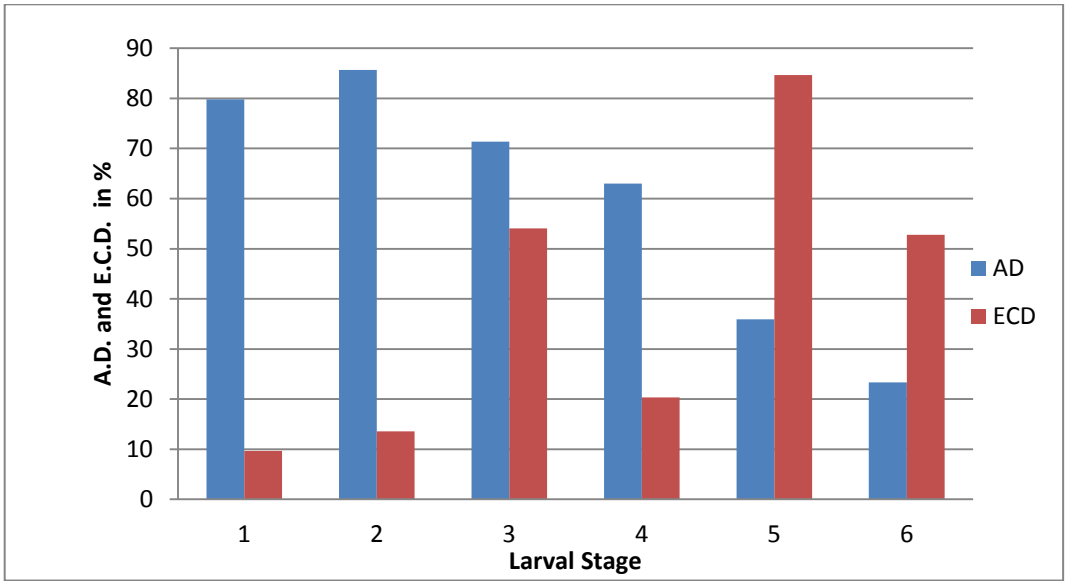

Fig-1: Graphical representation of A.D. and E.C.D. of *P.demoleus* under laboratory conditions.

Thus, from the present results this can be concluded that the approximate digestibility (A.D.) was maximum in the 2nd instar and the maximum conversion of digested food into tissue *i.e* (E.C.D.) was observed in the 5th instar. This results can be utilized for comparing the feeding efficiencies of oligophagous/ polyphagous insects. Based on the results on maximum food utility and its conversion into tissues in the form of weight gained by he insect, the preferred host can be judged.

REFERENCES

DAVEY, P. M. 1954. Quantities of food eaten by the desert locust, *Schistocerca gregaria* (Forsk.), in relation to growth. *Bull. Entomol. Res.***45**: 539-551.

SARADA, G. and GOPAL, K. 2013. Citrus Butterfly (*Papilio demoleus* Linnaeus) Biology and Management: A Review. *Research and Reviews: J. Agric.& Allied Sci,.***3**: 17-25

SANDHU, I. P. S. and SANDHU, G. S. 2011.Food consumption and indices of growth of *Plutella xylostella* (Linnaeus) on different cultivars of cabbage. *Journal of Insect Science* **24**: 88-93.

SATYAGOPAL, SUSHIL K.S.N., JEYAKUMAR P., SHANKAR G., SHARMA O.P., SAIN S.K.,BOINA D., LAVANYA N., VARSHNEY R., SUNANDA B.S., RAM ASRE, KAPPOR K.S., SANJAY ARYA, SUBHASH KUMAR, PATNI C.S.,CHATTOPADHYAY C., RAY P. K., KADAM U.K., BHATT J., DHAPURE S.R., EKABOTE S.K. THAKRE A.S., HALEPYATI A.S, PATIL, M.B., SREENIVAS, A.G., SATHYANARAYANA N. and LATHA, S. 2014. AESA based IPM package for Citrus. *NIPHM, Rajendranagar, Hyderabad, Ministry of Agriculture Government of India,* pp 53.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects, *Advances in Insect Physiology* **5**: 229-82.

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COPEPODS IN AYIRAMTHENGU MANGROVES AT KOLLAM, KERALA

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Key words: Cyclopoida, Calanoida, Herpacticoida, mangrove ecosystem

INTRODUCTION

Copepods constitute one of the major zooplankton communities occurring in all types of water bodies. Among micro crustacean planktonic, the copepods of orders Cyclopoida and Calanoida are one of the most representatives, being the largest biomass of the plankton community (ROCHA and MATSUMURA-TUNDISI, 1984). They are ubiquitos and small like the terrestrial ants (KRISHNAPILLAI, 1986). Copepods are much hardier and strongly motile than all other micro invertebrates with their tougher exoskeleton and longer and stronger appendages. They have long developmental time and a complex life history with early larval stages difficult to distinguish (GUNWATI, 2012) copepods are main grazers of phytoplankton. The oral appendages are flattened, multi lobed structures carrying long plumose setae. They feed voraciously taking in much more than they can digest and absorb. Much of they eat passed out undigested. There is however no waste of food since the excreta is consumed by other animals or broken down by bacteria and the nutrients contained in it recycled (KRISHNAPILLAI, 1986). So they play an important role in aquatic ecosystem.

Mangroves are specialized ecosystem developed along estuarine sea coasts and river mouths in tropical and subtropical regions of the world. The salt marshes and mangrove forests act like enormous filters. As water flow through this regions filter pollutants such as pesticides and heavy metals out of water as well as excess sediments and nutrients (USEPA, 1993). Present study was undertaken in the mangrove habitats of Ayiramthengu, a portion of Kayamkulam backwater. The mangrove ecosystem and its biological components are under the influence of both freshwater and marine condition and have developed a set of physiological adaptation to overcome problems of anoxia, salinity and frequent tidal inundations. This has led to the assemblage of a wide variety of plant and animals species of special adaptations suited to the ecosystem. The copepods assume a great ecological significance in mangrove ecosystem as this ecosystem is the feeding, breeding and nursery ground of many fin and shell fishes.

The copepod diversity of surface waters of various water bodies have been studied by GODHANTARAMAN (1994**),** and GUNWATI (2012). Studies made on copepods of Ayiramthengu mangrove are limited. A very few literature has been found on the plankton community of Ayiramthengu mangrove which is a part of Kayamkulam back water (MARY JOHN, 1958); AMINA *et al.* (2012). Thus the present study was carried out to record the diversity of copepods in this region.

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MATERIALS AND METHODS

Ayiramthengu mangrove ($9^0$6 to $9^0$8 N:76.28^0 to 76.29^0 E) is situated about 6 km west of Ochira town and the bank of Kayamkulam estuary, which is a narrow stretch of tropical backwater on the west coast of peninsular India. The mangrove covers 20 acre of area. This long chain of mangrove vegetation is the only extensive one left almost touched by man along Kerala coast. The area is mounted on east by Kayamkulam estuary on the west by the Kollam- Alappuzha water ways connected to Arabian sea on north and south by two canal. The flood water of Pampa and Achankovil rivers flow into the estuary. Ayiramthengu mangrove forest was declared an environmental hot sport after it was ravaged by the Tsunami 2004 and threatened with extinction.

The stations selected were having different ecological status

1. Station I is characterized by influx of fresh water into mangrove
2. Station II represent the luxuriant mangrove growth showing entangled the respiratory roots
3. Station III represent open area air tight and waves are predominates

To study the hydrological parameters, water samples were collected monthly (September, 2015-August, 2016) from three stations and brought to the laboratory and analyzed. Water temperature, pH, dissolved oxygen, carbon dioxide, salinity, hardness, TDS, BOD were carried out by standard procedure (APHA, 2005). The plankton samples collected by filtering 50 liter of water through standard planktonic net (60µ) and concentrated sample were preserved in 5% formalin in 100ml vial and identified with the help of standard keys.

RESULTS AND DISCUSSION

The mean values of various hydrological conditions recorded during the whole study period are given in Table-1. Water temperature, pH, and Dissolved oxygen are highest in station III, maximum value of free carbon dioxide and biological oxygen demand are seen in station II and highest value of salinity was shows in station III.

Table-1: Hydrological conditions of surface water at Ayiramthengu mangrove (mean value)

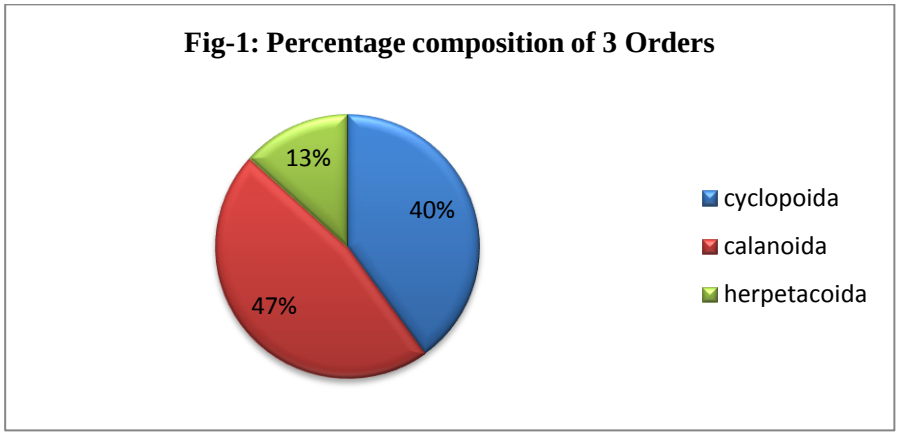
Parameters	**Sampling stations**		
	Station I	**Station II**	**Station III**
water temperature(^{0}c)	27.92	27.72	27.98
p^H	7.09	7.14	7.29
Dissolved oxygen(mg/l)	4.54	4.34	4.69
Free carbondioxide(mg/l)	5.68	6.07	5.46
Salinity	31.83	32.07	32.48
Biological oxygen demand	2.26	2.37	2.26

List of copepods recorded in study area is list in Table-2 and the percentage composition of three Orders are shows in fig-1. Which shows that during the study period 15 species of copepods belonging 3 Orders were noticed .Six species of Order Cyclopoida**,** 7 species of Order Calanoida and 2 species of Order Harpacticoida*.* List of copepods species recorded in Ayiramthengu mangrove (September, 2015 to August, 2016) showed that Order Calanoida was the most dominate group in Indian water as earlier reported by BIJU (2017). The percentage composition of three Orders depicted in Fig-2 also proveed this. They are probably the most ecologically significant animals at

the first consumer level of the marine plankton and are also the most prominent among the primary carnivores. Present study shows that the order Calanoida standing the dominant than the Cyclopoida and Herpacticoida.

Table-2: List of copepods species recorded in Ayiramthengu mangrove (Sept- 2015 to Aug 2016)

Order	copepod species	Station I	Station II	Station III
Cyclopoida	*Mesocyclops leuckarti* (Claus)	*	*	*
	M. hyalines	*	*	*
	M. aspericornis	*	*	*
	Thermocyclops sps	*		*
	T.crcessus	*	*	
	Eucyclops agilis	*	*	*
Calanoida	*Acarita centura* (Giesbrecht 1882)		*	*
	A. majar	*	*	*
	Diaptomes sps (Baird,1850)	*	*	*
	Paracalanus parvus(Calus)	*	*	*
	Acrocalanus longicornis (Giesbrecht)	*	*	*
	A.gibber (Giesbrecht)	*	*	*
	Ponetella danae ((Giesbrecht)		*	*
Herpacticoida	*Microsetella rosea*	*	*	*
	Longipedia weberi (Scott)	*	*	*



Copepods have been regarded as being good indicator of climatic trends and anomalies although they are generally considered as part of zooplankton community (BONNET and FRID, 2004). They are common in the coastal and estuarine waters, indicating nearness and linkage to the mangrove environments. Copepods are excellent candidate for the study of ecosystem response to climate variability because their life cycle is short. This characteristic makes copepod populations potentially capable to respond to environmental changes and reflect event-scale changes in environmental

conditions, hence provide early indications of biological response to climate variability (HAZEL and FATIMAH, 2015). Moreover, many copepod species are known to be bioindicators, whose presence or absence may represent the relative influence of different water types on ecosystem structures. The results found during the present study showed the presence of different species of copepods. The copepod diversity is closely related to water quality parameters and it also related the fish fauna of the water body.

REFERENCES

ALTAFF, K. 2004. A Manual of zooplankon, *UGC, New Delhi, Vol* 1, pp 145.

AMINA, S. 2012. Seasonal distribution of chlorophyceae in Kayamkulam back water. *Intern, J. Pharmacy & Life Sci*. **17**: 7-16.

APHA, 2005. Standard methods for the Examination of water and waste water. *2[1st] edn., APHA,AWWA, WPCF, Washington, D.C.,USA.*

Biju, A. 2017, A Hand Book of Marine Zooplankton, *Research Department of Zoology, St. Stephen's College, pathanapuram, Kerala,* pp2-15

BONNET, D. and C. FRID, 2004. Seven copepod species considered as indicators of water-mass influence and changes: results from a Northumberland coastal station *ICES Journal of Marine Science*, **61**: 485–491

DUSSAR, T. B. H. and DEFAYE, D, 1995. Copepoda: introduction to copepoda, *SPM Academic Publishing*, PP 6–7.

GODHANTARAMAM, N. 1994. Species composition and abundance of tintinnids and copepods in the pichavaram mangroves (South India), *Ciencias Marinas*, pp 371-391.

GONZÁLEZ, and MATSUMURA-TUNDISI, 2008. Size and dry weight of main zooplankton species in Bariri reservoir (sp, Brazil). *Brazilian Journal of Biology,* **68**: 69-75.

GUNWATI, V. ARAK, 2012. Copepod diversity of Tembhapury lake Aaurangabad region, m.s, India*. International Journal of Science and Research (ijsr).*

HAZEL**,** M**.** M**.** P**.** and FATIMAH**,** Md**.** Y**.,** 2015**.** Status of Planktonic Copepod Diversity in the Merambong Seagrass Meadow, Johor, Peninsular Malaysia, *International Journal of Ecosystem*, **5**: 39-43.

KATHIRESAN, K. and QASIM, S. Z. 2005. Biodiversity in mangrove ecosystem, *Hindustan Publishers, New Delhi*, pp 120

KRISHNAPILLAI, N. 1986, Introduction to Planktonology, *Himalaya publishing House*, *Mumbai*, pp5-79.

MARY JOHN, 1958. A preliminary study on Kayamkulam lake. *Bull.Central Res.Inst.University of Kerala.Trivandrum,* pp97-116.

GEORGE, J. P. and NAOMI, T. S. 2005. Mangrove ecosystems-a manual for the assessment of biodiversity, *CMFRI special publication No 83. Cochin*, pp36-82.

SUDARARAJ, V. and K. KRISHNMURTHY, 1975. Nutrient and plankton; backwater and mangrove environment*. In: Recent Researches in Esturine Biology, Hindustan Publishing Corp., New Delhi,* pp 273-290.

QASIM, S. Z. 2005. Zooplankton of some major estuaries of India, *J. Indian Ocean Stud.,* **1**: 439- 446

ROCHA, O. and MATSUMURA- TUNDISI, T., 1984. Biomass and production of *Argyrodiaptomus furcatus* a tropical calanoid copepod in broa reservoir, Southern Brazil. *Hydrobiologia*, **113**: 307-311.

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HEAVY METAL ANALYSIS IN FISH, *ETROPLUS MACULATUS* (BLOCH, 1795) OF KAREEPUZHA CHAAL, KAYAMKULAM, KERALA

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ABSTRACT: Aquatic populations interact with the physico-chemical and biological factors in their habitat. Any change in the habitat can induce stress on the life forms. Hence, analysis of water quality and heavy metal is to highlight the impact of pollution. The study was carried out during the month of January 2017 – June 2017 to evaluate the hydrological parameters and heavy metal content in fish species of Kareepuzha Chaal. The maximum and minimum ranges of hydrological parameters like temperature (27.2 -29.8 ^{0} c),pH (6.6 -8),DO (2.6 -1.6 mg/L), BOD (0.9 -1 mg/L)),Hardness (154 -192 mg/L)),salinity (0.5 -1.8 mg/L)),TDS (0.985 – 1.3 mg/L). The concentrations of heavy metals were analyzed using atomic absorption spectrophotometer. The average concentration of heavy metals present in the Site I follow this trend: Iron> Copper> Zinc> Chromium> Lead> Cadmium and that of Site II is Iron> Copper> Zinc> Chromium> Cadmium> Lead. The results revealed that the fish, *Etroplus macculatus* at Site I had 5.02 $\pm$ 3.25, 0.23 $\pm$ 0.01 , 0.02 $\pm$ 0.001, 0.02 $\pm$ 0.01, 0.01 $\pm$ 0.001, 0.01 $\pm$ 0.001 mg kg^{-1} as the mean concentration of Iron, Copper , Lead , Zink , Chromium , Cadmium, respectively while Site II had 5.8 $\pm$ 4.05 , 0.23 $\pm$ 0.01 , 0.02 $\pm$ 0.001 , 0.05 $\pm$ 0.02, 0.01 $\pm$ 0.001 , 0.01 $\pm$ 0.001 mg kg^{-1} as the mean concentration of these metals. The mean concentration of all heavy metal varied significantly among two sites ($P<0.05$). However the present study reports that the levels of all the heavy metals in fish samples were below the recommended maximum limit for human consumption.

Key words: Kareepuzha Chaal, Heavy metal, Hardness, Cadmium.

INTRODUCTION

Water is the medium in which all of life's chemical reaction occurs and it is an active participant in many of these reactions. Water is a most precious gift of nature, not only to the mankind, but to the whole organism living in different segments of the ecosphere (MANDAL, 2006). The backwaters of Kerala are a chain of brackish lagoons and lakes lying parallel to the Arabian Sea coast of Kerala state in southern India. 'The massive reclaimation of backwaters for agriculture, urbanization, housing, aquaculture and port construction has drastically affected the area's flora and fauna. Unbridled economic growth, a population explosion and increased human activities along coastlines are the most serious problems facing India's fragile ecosystems today and the backwaters are no exception to this. Alternative changes in physical, chemical and biological properties of water which may come harmful effects on human and aquatic organisms. The major hazardous metals of concern for India in terms of their environmental load and health effects are Lead, Mercury, Chromium, Cadmium, Copper and Aluminum. Their source is mostly anthropogenic- industrial activity, vehicles, etc. Structural and functional attributes of water, which may be categorized as physical, chemical, and biological have to be analyzed both qualitatively and quantitatively in order to have a comprehensive evaluation of the water quality. Kayamkulam Kayal, is located parallel to the Kerala coast from Sankaramangalam in the south (Kollam district) to Karthikapalli in the north (Alappuzha district). Since surface run off from various natural as well as man-made drainage systems dilutes the saline water in the lagoon, the Kayamkulam Kayal exhibits the character of an estuary. A small branch from the Achenkovil River passes southward and travels via Kareepuzha. This is known as the

Karipuzha Chaal which passes through Pathiyoor, Eruva, and finally reaches Kayamkulam. The water transports through this Chaal was the main route for businessman to carry their goods into Kayamkulam town. Aim of the study was to assess hydrological parameters and heavy metal content in water & fish samples from Kareepuzha Chaal.

MATERIALS AND METHODS

The area selected for the study during January-June, 2016 was the part of the Chaal. First site is the upstream of Kareepuzha Chaal and site II is the downstream part of Kareepuzha Chaal. The analysis of the physico - chemical characteristic of water samples were carried out following the standard procedures described in APHA (1995), TRIVEDI and GOEL (1986) and SAXENA (1998). Temperature of the samples was determined by using a mercury thermometer. pH of the samples were determined using a pH meter (Systronics, India) Dissolved oxygen (DO) of samples were tested using Winkler's iodometric method. Hardness of water samples were determined using Ethylene Diamine Tetra Acetic Acid (EDTA) method. Salinity was noted by Mohr method (MOHR, 1856). Methodology involves water samples were collected in cleaned and dried Plastic bottle for the Analysis of heavy metals. The concentration of heavy metals in water samples were determined after acid digestion to 500 ml water sample, 5 ml concentric acid was added and digested in a digestion chamber made up to 50 ml volume and filtered. Heavy metal content (Cu, Zn, Cd, Cr, Pb and Hg) was determined using an Atomic absorption Spectrophotometer (Chemittco AA, 203).

The commonly available fish, *Etroplus maculatus* was selected for the study. Fishes were collected with the help of local fisherman from two sampling sites of Kareepuzha Chaal. Healthy fishes of length 15 to 20 cm and weight 95 to 115 gm were selected for the study. Fishes were transported to the laboratory for the analysis. The fish samples were dried using oven to remove the moisture content, after which they were homogenized to a powdered form using ceramic mortar and pestle. Sample preparation was done very carefully without contamination. All glass wares used were washed and rinsed, rinsed again with distilled water and then washed in 10% HCl. Wet digestion (partial) was used. One gram of the dry ground and sieve sample was weighed into a 100 ml beaker. Concentrated HNO_3 and HNO_4 were added in the ratio of 2:1 and was covered with a watch glass and placed on a hot plate in a fume cupboard where it was heated to near dryness till there was a change of colour to white. It was allowed to cool before leaching the residue with 5 ml of 20% HNO_3. The filtration was done using acid wash filter paper (Whatman) and finally the volume was made up to 20 cm^3 with distilled water. A blank/control was prepared similarly without the addition of the sample (AOAC, 2004). Microsoft Excel (MS Excel) was used to analyze data. Single Factor Analysis of Variance (ANOVA) was performed to compare the group means and statistical significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

The physical and chemical characteristics of water in any locality exert their influence on the occurrence and abundance of the biological components. The mean temperature of Site I was 28.55˚C and Site II as 28.07˚C (Tables 1 & 2). According to GOLDMAN and HORNE (1983), temperature variations are much less in tropical regions than in temperate regions. In the present study mean pH value of water samples in Site I was 7.52 and that of Site II were 7.18 (Tables 1 & 2). This nature of the water may be due to the percolation of leachate from many dumping sites near the water body. Solid waste contains many types of materials which is acidic or alkaline in nature. This can be affected to the nearest ecosystems. Similar values are got by SUNIL (2004) during his study on the water bodies near Kureepuzha waste dumping site. The mean value of

Dissolved Oxygen in water sample of Site I was 2.033 mg/L and that of Site II was 2.167mg/L (Tables 1 & 2).

This clearly indicates the effect of waste dumping on the water body. Studies by NAIR and AZIZ (1987) showed wide fluctuations in the dissolved oxygen concentration in the Ashtamudi Lake. The maximum concentration of DO was recorded on the month of June in both sites (Site I - 2.5 mg/L and Site II- 2.6 mg/L). Dissolved Oxygen affects the solubility and availability of many nutrients and therefore, productivity of aquatic ecosystem (WETZEL, 1983). The average BOD content in the water in Site I was found to be 0.783mg/L and Site II was 0.617mg/L. Leaching from the waste disposal sites enrich the water with the nutrients.

Table-1: Physico-chemical parameters of the study site I

Parameter	**Minimum**	**Maximum**	**Mean ± SD**
Salinity	0.5	1.8	1.05 ± 0.48
pH	7.2	8	7.52 ± 0.35
CO_2	0.1	0.3	0.017 ± 0.008
Hardness	164	194	177.5 ±11.55
Dissolved oxygen	1.6	2.5	2.03 ± 0.31
BOD	0.2	1.2	0.78 ± 0.40
Temperature	27.2	29.8	28.55 ± 1.09
TDS	0.46	1.3	0.98 ± 0.30

Table-2: Physico-chemical parameters of the study site II

Parameter	**Minimum**	**Maximum**	**Mean ± SD**
Salinity	0.5	1.8	1.05 ±0.46
pH	6.6	7.9	7.18 ±0.47
Co_2	0.01	0.03	0.015 ±0.008
Hardness	154	182	169 ±10.48
Dissolved oxygen	1.9	2.6	2.17 ±0.25
BOD	0.1	1.1	0.62 ±0.44
Temperature	27.5	28.6	28.07 ±0.37
TDS	0.58	1.3	0.98 ±0.26

In the present investigation mean value of hardness in Site I was 177.5 mg/L and that of Site II was 169 mg/L (table 1 and 2). High hardness is mainly due to the saline nature. Higher hardness in the waste water of Vellayani fresh water lake has been reported by KRISHNA KUMAR (2002) also reported higher hardness in Paravoor Lake. The mean salinity in Site I and Site II are same (1.050) (Tables 1 & 2). Salinity in the industrial areas was higher than the other sites of the river. This observation supports the study of JOSEPH *et al.* (1984) in Periyar Lake. Total dissolved solids in Site I was 0.985mg/L and that of Site II was 0.977mg/L. The difference between Site I and the other site was statistically significant. In the present investigation mean value of Carbon dioxide (CO_2) in Site I was 0.017 mg/L and that of Site II was 0.015mg/L (Tables 1 & 2).

Monthly variation in the heavy metal concentration in water sample

Trace metals in water samples from the two different sites of Kareepuzha Chaal in Kayamkulam backwater were analysed. To know more about the pollution and contamination, a comparative study on heavy metals (mainly Iron, Copper, Lead, Zinc,

Chromium and Cadmium) is made during January 2017-June 2017. The average concentration of heavy metals present in the Site I follow the trend: Iron> Copper>Zinc> Chromium>Lead> Cadmium (figure 1). The average concentration of Iron in the Site I was 4.04mg/L; Copper, Zinc, Chromium, Lead and Cadmium was 0.065mg/L, 0.062mg/L, 0.027mg/L, 0.020mg/L and 0.018mg/L, respectively. In Site II, the average concentration of heavy metals follows the trend: Iron>Copper>Zinc> Chromium> Cadmium>Lead (Fig.2). The average concentration of Iron was 3.495mg/L. Copper, Zinc, Chromium, Cadmium and Lead was.036mg/L, 0.035mg/L, 0.023mg/L. 0.018mg/L and 0.012mg/L, respectively.

The present study reveals that Iron concentration was comparatively higher in the Site I (6.1 mg/L) than the Site II (5.4 mg/L) in the month of January. In accordance with the work of ONIYE *et al*. (2002) in their work in Zaria dam, Nigeria, they reported higher concentration of Iron in the rainy season. Maximum amount of Copper was observed on the month of March in both the sites (Site 1 -0.09 mg/L and Site 2-0.06 mg/L). Copper concentration was gradually decreased on the months of May & June in the Site II (May-0.02 mg/L mg/L and June-0.008 mg/L). There was no 42 change in concentration in site I (May-0.05 mg/L and June-0.06 mg/L). Result of the present study reveals that Site I (mg/L) is more polluted than Site II (mg/L). The highest concentration of Zinc was seen on the month of March 2017 in Site I (0.08 mg/L). Due to summer drought and pre-monsoon, Site II showed decreased concentration from March to June. (March-0.04 mg/L, April-0.03 mg/L, May-0.02 mg/L, June-0.01 mg/L).The average of Zinc in Site I was (0.062 mg/L) and that of Site II was (0.035 mg/L). From this observation we can conclude that both the sites having the concentration of Zinc within the permissible limit recommended by BIS. The major causes of Zinc emission are the anthropogenic sources specifically mining operations (HUTTON and SYMON, 1986; NRIAGU, 1989).

Site I and Site II show the value of Lead is lower than the desirable limit. In Site I the months of January and March (0.03 mg/L), February and April (0.02 mg/L), May and June (0.01 mg/L) have same concentration of Lead. Similarly in the Site II January and March (0.02 mg/L) and February, April and May (0.01 mg/L) having same concentration. The least concentration of Lead is found in the month of June in Site II (0.001 mg/L). Analytical data reveals that in all the water samples, Chromium concentration is within the permissible limit of 0.05 mg/L prescribed by Bureau of Indian Standard. In India, the Chromium level in underground water has been witnessed to be more than 12 mg/L and 550–1,500 ppm/L. Both the sites show maximum concentration of Chromium in the month of April (0.04mg/L). From the arrival of monsoon Chromium concentration is decreased in Site II (May-0.02 mg/L and June- 0.01 mg/L). Average of Chromium concentration gives the idea that Site I (0.027 mg/L) having more content than Site II (0.023 mg/L). Chromium is released into the environment through sewage and fertilizers (GHANI, 2011). In the study, the data reveals that all the water samples of both the sites showed higher cadmium concentration than that of permissible limit. This is due to the influx of solid wastes. Cadmium contamination of water may be the result of discharge from industrial effluents, old plumping and house hold sewages. Both the sites have same concentration of Cadmium in average. In Site I, minimum concentration of Cadmium was seen in the months of January and June (0.01 mg/L) while same concentration of Cadmium was found in the months of February, March and May (0.02 mg/L). It is observed that month of April shows maximum concentration (0.03 mg/L) in Site I. In Site II, January, February and April (0.02 mg/L), March and June(0.01 mg/L) showed same concentration. Maximum concentration of Cadmium was seen in the month of May (0.03 mg/L) in Site II. In the US, more than 500,000 workers get exposed to toxic Cadmium each year as per the Agency for Toxic Substances and Disease Registry (BERNARD, 2008).

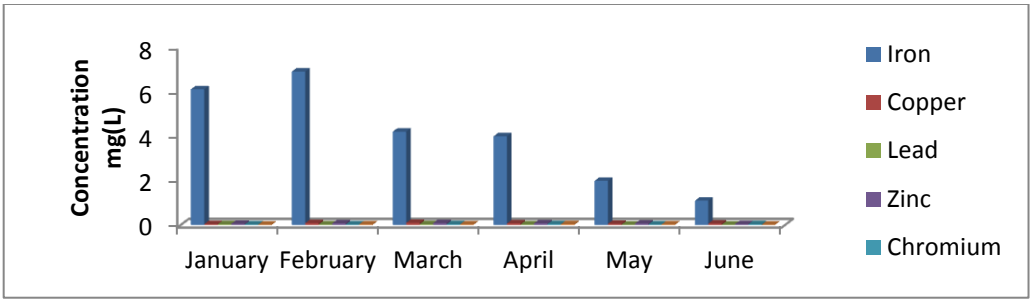

Fig. 1: Monthly Variation of Heavy metals in Water Sample in Site I

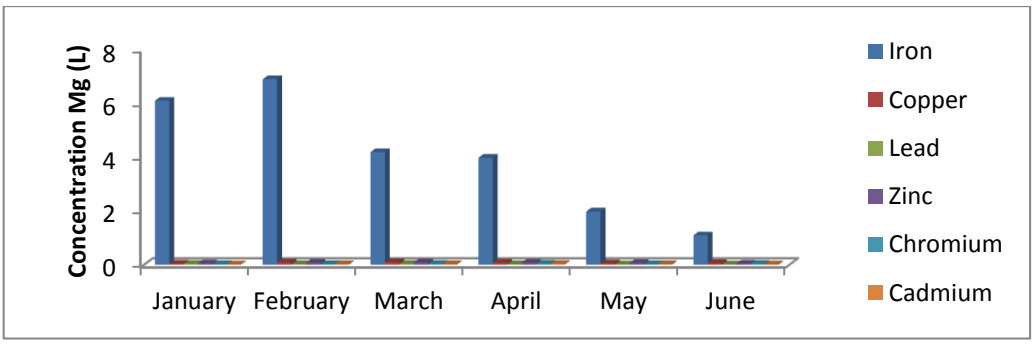

Fig. 2: Monthly Variation of Heavy metals in Water Sample in Site II

Table-3: Monthly variation in the heavy metal concentration in fish sample

Study Sites	Iron	Copper	Lead	Zink	Chromium	Cadmium
Site I	0.3 ± 0.02	0.23 ± 0.01	0.02 ± 0.001	0.02 ± 0.01	0.01 ± 0.001	0.01 ± 0.001
Site II	0.4 ±0.05	0.03 ± 0.01	0.02 ± 0.001	0.05 ± 0.02	0.01 ± 0.001	0.01 ± 0.001

The mean concentration of the heavy metals in the fish samples is presented in Table 3 and a comparison of mean value of metal concentration (mg kg^{-1}) with maximum limits (Reference Value) is presented in table 4. The mean value of iron ranged between 0.3 ± 0.02 to 0.4 ± 0.05. The higher concentration was noted in fishes from Site II. The accumulation level of Copper ranged between 0.23±0.01 to 0.03±0.01 with the higher concentration reported in Site I and the lower concentration was in Site II. The concentration of lead ranged between 0.02±0.001 with the higher accumulation noted in Site I and the lower concentration was in Site II. The overall mean concentration was significantly ($P<0.05$) higher in Site I. Similarly, the concentration of Cadmium and Chromium ranged between 0.01±0.001. There was no significant variation among two sites. The mean concentration of Zinc ranged between 0.05±0.20 to 0.02±0.01 with the higher concentration recorded in site II and the lower concentration was in Site I.

Aquatic environment becomes polluted with heavy metals which is drastically increased worldwide attention and under certain favourable environmental conditions, fish may accumulate large amounts of some of these heavy metals in their tissues from

the sediment and water. Heavy metal pollutants are currently considered to be some of the most toxic, persistent and widespread contaminants in estuarine systems in the sense that dissolved or suspended metals become available to plankton, nekton, and benthic filter and deposit feeders (JOSHI and BALASUBRAMANIAN, 2010). According to Olowu, *et al*., (2010), non-biodegradable compounds are the most dangerous among the pollutants due to their innate ability to constantly remain within the ecosystem. The mean concentration of iron in fish sample was 0.3 $\pm$ 0.02 at Site I, while Site II had 0.4 $\pm$ 0.05 mg kg^{-1}.

Table-4: A comparison of mean of total metal concentration (mg kg^{-1}) with maximum limits

Heavy metal	Mean of total concentration		Maximum limit	References
	Site I	Site II		
Iron	0.3 $\pm$ 0.02	0.4 $\pm$ 0.05	0.5	WHO (2006)
Lead	0.02±0.001	0.02±0.001	0.50	FAO (1983)
Copper	0.23±0.01	0.03±0.01	3.0	WHO (2006)
Cadmium	0.01±0.001	0.01±0.001	0.50	FAO (1983)
Chromium	0.01±0.001	0.01±0.001	12.00 – 13.00	USFDA (1993a)
Zinc	0.02±0.01	0.05±0.02	30.00	FAO (1983)

These values may below the maximum limit 5.6 mg kg^{-1} recommended by WHO (2006). Accumulation of lead in fish samples were below the FAO (1983) guideline of 0.50 mg kg-^{1} in food fish. The mean value of lead was also below 0.02±0.001 mg kg-^{1} in Site I and Site II. This is lower than that the values recorded by the study of KHOSHNOUD *et al*., (2011) and SAFAHIEH *et al*., (2011). Monthly flactuation in the concentration noted that Lead accumulated mainly in the tissues of the fish species at site II in July. This may be due to heavy rainfall in this month. The concentration of Copper in the fish samples were below the FAO (1983) maximum limit of 30.00 mg kg^{-1} in food fish. The mean concentration was in the range of 0.23±0.01mg kg^{-1} at Site I and 0.03±0.01 at Site II. This is similar to the findings of AKPANYUNG *et al.* (2014), who worked on *C. nigrodigitatus*. There was a significant monthly variation in the concentration among the two sampling sites. The mean concentrations of Cadmium and Chromium were below the FAO (1983), of 0.50 mg kg^{-1} and range of 12.00 – 13.00 mg kg^{-1} guideline for food fish. This result is similar to findings by KHOSHNOUD *et al*., (2011) and AKPANYUNG *et al.* (2014) that worked on *C. nigrodigitatus*. HANSER *et al*. (2009) reported that high doses of Cadmium can Lead to kidney failure, damage to testicles and liver. The concentration level Zinc was very high at sites during the study period, the mean value was between 0.02±0.01 to 0.05±0.02, was below the 30.00 mg kg-^{1} FAO (1983) guideline for food fish.

CONCLUSION: The present baseline data on physico-chemical parameters and heavy metal concentrations in water indicates that the local environment is totally different from any known natural ecosystem, and it cannot be compared or applied to other areas. Analysis of heavy metals in water sample and fish sample shows that the levels of Iron, Copper, Cadmium, Lead, Chromium and Zinc from the two sites are all below the maximum limits specified by the international authorities like WHO (2008), FAO, USFDA. Improper disposal of solid wastes, industrial effluents, house hold wastes causes severe contamination of the Kareepuzha chaal. The concentration of heavy metals and other water quality parameters undergo seasonal and monthly changes. This water was little more polluted and not recommended as for human consumption. There is a possibility of bioaccumulation of metals in fish. Proper methods of waste disposal

should be adopted and other anthropogenic activities should b continuously checked. The prevention of agricultural runoff into the water system is also an adequate step to preserve a healthy aquatic environment and in a whole protect the life of people who consume these fishes. Proper action should be taken to avoid future contamination of the water body and subsequently the fish living in that area, since their synergistic effect can still cause a very harmful health problem.

REFERENCES

AKPANYUNG, E. O., UDOUDO, M. E., EKAM, I. A., AND NGOZI, O. A. 2014. Levels of heavy metals in fish obtained from two fishing sites in Akwa Ibom State, Nigeria. *African Journal of Environmental Science and Technology.* **8(7)**: 16 – 421.

AOAC, 2004. *Official Method of Analysis of the Association of Official Analytical Chemists, 15th edition, Washington DC. USA*, pp1050.

APHA (American Public Health Association) 2012. *Standard methods for the examination of water and wastewater (22th ed.). Washington, DC: American Public Health Association*.

BERNARD, A. 2008**.** Cadmium & its adverse effects on human health. *Indian J. Med. Res.* **128**(4): 557–64.

HUTTON, M AND SYMON, C .1986. The Quantities of Cadmium, Lead, Mercury and Arsenic entering the U.K. Environment from Human Activities. *Sci. Total Environ.* **57**: 129-150.

FAO 1983. Compilation of legal limits for hazardous substances in fish and fishery products. *Publication of Food and Agriculture Organisation*. *FAO Fish Circ*., **464**: 5-100.

GOLDMAN, C. R., AND A. J. HORNE. 1983. Limnology. *McGraw-Hill Book Co., New York, 464p.*

HANSER, R. AND MARRION, H. 2009. Articles on natural medicine, "The dangers of heavy metals. *Environ. Health Perspect*., **89**: 91-94.

JOSHI, U.M. AND BALASUBRAMANIAN, R. 2010. Characteristics and Environmental mobility of trace elements in urban runoff. *Chemosphere*, **80**: 310-318.

KHOSHNOUD, M. J., MOBINI, K., JAVIDNIA, K., HOSSEINKHEZRI, P. AND JAMSHID, K. A. 2011. Heavy metals (Zn, Cu, Pb, Cd and Hg) contents and Fatty Acids Ratios in two fish species (*Scomberomorus commerson* and *Otolithes ruber*) of The Persian Gulf. *Iranian Journal of Pharmaceutical Sciences,* **7**(3): 191-196.

MANDAL .2006. A summary of the biochemistry of compounds in ground water. *J. Hydrology* **27**(1-2): 155–167.

NRIAGU, J. O. and PACYNA, J. 1988. Quantitative assessment of worldwide contamination of air, water and soil by trace metals, *Nature*, **333**: 134-139.

OLOWU, R. A., AYEJUYO, O. O., ADEWUYI, G. O., ADEJERO, I. A., DENLOYE, A. B., BABATUNDE, A. O., AND OGUNDAJO, A. L., 2010. Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry lagoons, Lagos, Nigeria. *E-Journal of Chemistry*, **7**(1): 215-221.

SAFAHIEH, A., RONAGH, M. T., MONIKH, F. A., SAVARI, A., AND DORAGHI, A. 2011. Heavy metal concentration in belaner's croaker fish, *Johnius belangerii* from petrochemical waste receiving estuary in Persian Gulf, Iran. *2nd Intern. Conference on Environmental Engineering and Application; IPCBEE vol. 17. IACSIT, press, Singapore.*

SRIVASTAV. A. K, RAI. N. SUZUKI, D. MISHRA AND S. K. SRIVASTAV (2013). Effects of lead on the plasma electrolytes of a freshwater fish, *Heteropneustes fossilis*. *International Aquatic Research*, **5(4)**: 1-7.

TRIVEDY, R.K., AND GOEL, P.K., 1984. Chemical biological methods for water Pollution studies. *Env.Pub.India,* pp.104.

WHO (World Health Organization). 2006. *Guidelines for Drinking Water Quality. First Addendum to the Third Edition Volume 1. Wikipedia, Metal toxicity, Available At Website: www.google.com .2013.*

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PERFORMANCE OF ONION VARIETIES AGAINST THRIPS (*THRIPS TABACI* LINDEMAN) INCIDENCE AT BARGARH, ODISHA

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ABSTRACT: A replicated field trial was conducted in farmers' field at village Ludupali of Ambabhana block in Bargarh district, Odisha to evalute the performance of 7 onion cultivars including a local variety, against thrips (*Thrips tabaci,* Lindeman) incidences during rabi 2013-14. The study revealed that. at 90DAS Nasik Red and Agri Found Light Red recorded thrips population of 86.52 and73.80/plant, respectively. The per cent leaf damage was recorded both at vegetative and reproductive phases of the crop. The thrips incidence started to build-up during vegetative stage of the crop and the per cent leaf damage varied from 4.75 to 28.32. The lowest per cent leaf damage was recorded in AFLR which was followed by Bheema Super (5.36) and significantly different from the remaining treatments. The highest bulb yield of 234.00 q/per ha was recorded in AFLR which was significantly superior to all other varieties. The next best treatment to record higher yield was Bheema Super and Red Ball variety recorded the lowest yield (190q/ha). However, control treatment recorded significantly higher yield over Red ball and N-53. The cost economics of different treatment was calculated by taking onion sale price as Rs.10,000/ton and Rs. 35,000 as cost of production per hectare excluding cost of insecticides . The net profit was highest in AFLR (Rs.1,99,000/ha), followed by Bheema Super (1,90,000/ha) and lowest profit was recorded from Red Ball variety (Rs. 1,55,000/ha). The Benefit Cost ratio was highest in AFLR (1:6.88), followed by Bheema Super (1:6.42), AFDR (1:6.32), Bargarh local (5.99) and Black Beauty(1:5.71)

Key words: Onion thrips, benefit cost ratio

INTRODUCTION

Insect pests are one of the important factors responsible for reduction of onion yield. The major insect pests attacking onion crop are thrips (*Thrips tabaci* Lindeman), aphids (*Myzus ascalomicus* Doncaster), cutworm (*Agrotis segetum* Schiff), leek moth (*Acrolepiopsis assectella* Zeller), onion maggot (*Delia anticua* Meigen), bean seedfly (*Hylemya platura* Rond.), seed corn maggot (*Delia platura* Meigen), leaf beetles (*Lilioceris* spp), bulb mites (*Rhizoglyphus callae* Ondemans), gall mites (*Aceria tulipae* Keizer), snails and slugs. Onion thrips, *Thrips tabaci* Lindeman has been identified as a pest of national importance in India. The pest is active throughout the year and found on onion and garlic from November to May. The infested leaves get twisted and develop white patches. The crop may suffer heavy losses even up to 50% (RAHMAN and BATRA, 1945). To combat the insect pests, farmers are solely dependent on pesticides. Onion grower typically applies insecticides weekly, resulting in 9-12 insecticides applications per cropping season. Besides the economic cost, the health and environmental problems that arise from such intensive application of insecticides, this practice is conducive to the development of insecticide resistance, which is an increasing problem with onion thrips (SHELTON *et al*., 2006). Other alternative strategies are also adopted for managing this pest. *Thrips tabaci* still causes significant yield loss despite

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decades of research on control strategies worldwide (Lewis, 1997). *Thrips tabaci* feeding can reduce onion bulb weight (FOURNIER *et al*., 1995; RUEDA *et al*., 2007; DIAZ MONTANO *et al*., 2008; 2010). Young onion plants are more susceptible and prone to be killed by high *T. tabaci* infestations and relatively insensitive to thrips feeding late in the season. Host plant resistance is considered as cornerstone of integrated pest management (IPM) (PANDA and KHUSH, 1995 and KENNEDY, 2008) and may offer a long-term solution to *T. tabaci* control and reduce the use of insecticides, which would lower environmental hazards and minimize the evolution of resistance to insecticides. Hence, in the present investigation, seven improved cultivars of onion including one local check were tested for their reaction to thrips as well as yield at the agro climatic region prevailing at Bargarh district of Odisha state.

MATERIALS AND METHODS

Seven improved varieties of onion including one farmers preferred variety as detailed in Table-1, constitutes seven treatments which were practiced in 8 farmers' field in Ludupalli village of Bargarh district located at Ambabhena block. All the farmers accepted agronomic practices except plant protection were followed for raising the crop during Rabi season of the year 2013-14. Visual observations were made on thrips population on five plants at 15 days interval on all the cultivar. Observations were also made on leaf damage both at vegetative phase and reproductive phase. The per cent leaf damage is calculated by using the following formula

$$\text{Per cent leaf damage} = \frac{\text{Number of damaged leaves (5 plants)}}{\text{Total number of leaves (5 same plants)}} \times 100$$

The thrips population was correlated with leaf damage. The number of thrips was counted at 15, 30, 45, 60, 75 & 90 days after sowing in ten randomly selected plants from each treatment plot of each replication, leaving the border rows. The yield at harvest was also recorded. Then the yield was converted on hectare basis and subjected for statistical analysis. The cost economics such as net profit, net gain, and benefit cost ratios of each treatment were worked out by using the formula

Net profit = Gross income – Cost of cultivation
Net gain= Net profit in treatment - net profit in control

$$\text{Benefit cost ratio} = \frac{\text{Gross income}}{\text{Cost of cultivation}}$$

Visual observation was made on the leaf colour of different varieties and categorized as light green, green and dark green. Further, graded 1 for light green, 2 for green, and 3 for dark green to facilitate statistical analysis. The above characters were correlated with thrips population. The whole experiment was planned in randomised block design and yield as well as correlation coefficient was calculated as per the procedure of GOMEZ and GOMEZ (1984).

RESULTS AND DISCUSSION

Seven onion cultivars (Table-1) were tested against onion thrips infestation at village Ludupali of Ambabhena block of Bargarh district of Odisha under field condition during 2012-13 *Rabi*/Summer season. The thrips present on onion was identified as *Thrips tabaci*. Thrips population was counted at every 15 days interval throughout the season. Initially (15 days after sowing DAS) the average population varied from 10 to 20 per plant. The lowest thrips population of 10/plant was recorded on Red ball variety and

that of highest was on AFLR variety. There was considerable change in the thrips population at 30 DAS. The lowest thrips population was recorded on AFLR (26.23 thrips/plant) which was at par with Black Beauty (28.35thrips/plant) and significantly different from Nasik Red and local variety. Almost similar population build-up was observed, when crop was at 45 DAS except Nasik Red. However, there was slight deviation as the AFLR recorded significantly lowest thrips population and differed significantly with all the other varieties in the trial. AFDR recorded next lowest population and was at par with the Bheema Super and significantly different from remaining varieties. However, at 60 DAS, there was distinct difference between varieties in supporting lower and higher population. Nasik Red (59.86 thrips/plant) and Black Beauty (52.40 thrips/plant) supported significantly highest population and lowest population was recorded from AFLR,. Similar trend in thrips population was continued up to crop maturity. At 90DAS, Nasik Red and AFLR recoded thrips population of 86.52 and 73.80/plant, respectively. All the remaining varieties supported higher thrips population (more than 50 thrips/ plant) throughout the season (Table-1).

The per cent leaf damage was recorded both at vegetative and reproductive phase of the crop. The thrips incidence started to build-up during vegetative stage of the crop and the per cent leaf damage varied from 4.75 to 28.32. The lowest per cent leaf damage was recorded in AFLR which was followed by Bheema Super (5.36) and significantly different from remaining treatments. Remaining varieties recorded higher leaf damage from the vegetative stage itself. The highest leaf damage of 23.35 was recorded in N-53, which remained statistically different from all the other varieties. The per cent leaf damage at reproductive stage varied from 29.35 to 86.75. The per cent leaf damage was not below 25 in any of the cultivars during the season. The lowest per cent leaf damage of 29.35 was observed in AFLR, and differed significantly with rest of the genotypes. All the remaining varieties recorded more than 70% leaf damage except AFDR and Bheema Super. The onion varieties are categorised under two types of foliage such as glossy and non glossy. The majority of varieties such as Bheema Super, AFDR and AFLR were having glossy foliage. The remaining varieties in the trial recorded non glossy foliage (Table-1).

There were three categories of foliage color such as light green (LG), green (G) and dark green (DG). Three were having green color foliage such as Bheema super, N-53 and AFDR, where as two varieties were recorded light green and rest were recorded dark green foliage. The leaf thickness of AFLR and Bheema Super at middle was recorded 18.35 and 17.35 mm without press and 2.01 as well as1.95 mm at middle with press for the same varieties, respectively. All others recorded lower value for this character. There was significant positive correlation between foliage colour and thrips population (0.860). The correlation coefficient between per cent leaf damage at both the stages and thrips population was found as significant at both 5% and 1% level (Table-2). The bulb yield was recorded in all the experimental plots and computed on hectare basis. The highest bulb yield of 234.00q/ ha was recorded in AFLR which was superior to all other varieties and differed significantly from all the other treatments. The next best treatments to record higher yield was Bheema Super and Red Ball variety recorded the lowest yield(190q/ha). However, control recorded significantly higher yield over Red ball and N-53. The cost economics of different treatment was calculated by taking onion price as Rs.10,000/ton and Rs. 35,000 as cost of onion production per hectare excluding cost of insecticides. The seed cost causes differential in cost of production. The net profit was highest in AFLR (Rs.199000), followed by Bheema Super (Rs.1,90,000) and lowest profit was recorded from red ball variety(Rs. 155,000). The benefit cost ratio was highest in AFLR (1: 6.88), which was followed by Bheema Super (1:6.42), AFDR (1:6.32), Bargarh local (5.99) and Black Beauty (1:5.71) (Table-3).

Table–1: Performance of onion varieties on thrips (*Thrips tabacci* lindman) incidence of Rabi Onion during Rabi 13-14 at Bargarh, Odisha.

S. No.	Name of the variety	No of thrips / plant *						Percentage foliage damage **		Foliage type	Foliage colour	Leaf middle thickness (mm) ***	
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Vegetative stage	Reproductive stage			without press	with press
1.	Bheema Super	10.11 (3.17)	28.35 (5.32)	36.18 (6.00)	42.35 (6.50)	50.25 (7.08)	59.31 (7.70)	5.36 (13.31)	34.25 (39.92)	GF	G	17.37±1.35	1.95±0.06
2.	N-53 (Nasik Red)	19.00 (4.58)	31.23 (5.58)	51.95 (7.33)	59.36 (7.89)	68.12 (8.33)	86.52 (9.36)	23.35 (28.86)	70.75 (57.23)	NGF	G	16.75 ±1.97	1.85 ±006
3.	AFDR (Agri found dark red)	13.52 (3.67)	28.35 (5.32)	35.39 (5.99)	43.55 (65.99)	55.15 (7.42)	61.35 (7.83)	17.31 (24.58)	35.35 (36.45)	GF	G	15.35 ± 1.83	1.73±0.07
4.	AFLR (Agri found light red)	11.52 (3.52)	26.23 (5.38)	32.12 (5.66)	41.35 (6.44)	49.50 (7.03)	52.35 (7.23)	4.75 (12.52)	29.35 (32.77)	GF	LG	18.35±2.05	2.01±0.08
5.	Red Ball	10.00 (3.32)	29.85 (5.55)	38.15 (6.26)	48.29 (7.03)	56.28 (7.56)	62.39 (7.95)	23.35 (7.95)	86.75 (25.90)	NGF	DG	11.35±2.05	1.55±0.06
6.	Black Beauty	10.12 (3.80)	28.15 (5.40)	39.25 (6.35)	52.40 (7.23)	53.22 (7.36)	73.80 (8.59)	25.55 (30.33)	75.52 (60.23)	NGF	DG	12.85±1.85	1.70±0.06
7.	Local	14.50 (3.80)	30.11 (5.48)	40.23 (6.34)	48.50 (6.94)	54.55 (2.3)	62.30 (7.89)	28.32 (32.14)	72.35 (58.24)	NGF	LG	11.85±1.73	1.78±0.06
	SEm (±)	0.621	1.01	1.22	1.28	1.75	1.31	1.81	3.25	-	-	0.83	-
	CD (P=0.05)	1.281	2.084	2.518	2.641	2.373	2.683	3.735	6.708	-	-	1.713	-

Values in parenthesis are transferred values:
* Square root transformation
** Are sure transformation
*** Values are mean of 5 plants ± S.D.

G.F. – Glossy foliage
VGF - Non Glossy Foliage
G - Green, LG - Light Green
DG - Dark Green.

Table - 2: Correlation between biophysical characters of onion verities and thrips population.

S. No.	Host plant characters	Thrips population per plant
1.	Percentage foliage damage at veg stage	0.869 **
2.	Percentage foliage damage at rep stage	0.897 **
3.	Leaf thickness at middle (mm) without press	0.533 *
4.	Leaf thickness at middle with press	-0.015
5.	Leaf colour	0.850 **

* Significant at P= 0.05 level; ** Significant at P= 0.01 level.

Table-3: Yield & economics of different onion varieties grown during Rabi 2013-14 at Bargarh, Odisha.

S. No.	Name of the var.	Yield (q/ha)	Gross income (Rs/ha)	Cost of cultivation (Rs/ha)	Net profit Rs/ha	Net gain over control (Rs/ha)	Benefit Cost Ratio
1.	Bheema Super	225.0	2,25,000	36,000/-	1,90,000	28,000	6.42
2.	N 53 (Nasik red)	192.0	192000/-	350000/-	1,57,000	5,000	548
3.	AFDR	215.0	215000/-	34000/-	1,80,000	28,000	6.32
4.	AFLR	234.0	2,34,000/-	34000/-	1,99,000	27,000	6.88
5.	Red ball	190.0	190,000/-	35000/-	1,55,000	7,000	5.42
6.	Black Beauty	202.0	202000/-	35000/-	1,67,000	5,000	5.71
7.	Local	197.0	197000/-	34000/-	1,62,000	-	5.99
	SEm (±)	3.031	-	-	-	-	-
	CD @ 5%	6.25	-	-	-	-	-

Cost of onion cultivation (Rs/ha) = Rs. 35,000; Excluding pesticides

Initially at vegetative stage of the crop leaf damage was very low and as such there was no much difference. However, at reproductive stage of the crop damage was clear cut and eye catching. BHANGALE and JOI (1985) screened 74 onion cultivars and reported that all were susceptible, but Gujarat Sel-1003 had the lowest population of thrips. Among the 34 onion cultivars evaluated five cultivars such as N 2-4-1, Se1-104, Ratnar, Sel-171, HR Browand Sel-202 were highly resistant to natural infestation by *T. tabaci* having fewer than 35.7 thrips/5 plants (SINGH *et al*., 1977). PAWAR *et al.* (1987) screened 64 genotypes for resistance to thrips and observed that Kalyanpur Red Round, Udaipur103, Stedgol, White, Mathewad1, Shirwal-2, White Creole and Kagal-2 were resistant. PATIL *et al*, (1988) evaluated 28 onion cultivars and results confirmed that three cultivars, N-257-9-7, Hissar-2 and N-2-4-1 were resistant to thrips. Similarly among 61 onion genotypes PBR-3, PBR-4, VL-1, No.18, No.19 and Pusa Ratnar were less preferred by thrips (BRAR *et al*., 1993). Pusa Red had the lowest infestations while Patna Red and Arka Niketan had the highest infestations in both seasons (SINHA *et al*., 1995). MALIK *et al.* (2003) evaluated six onion varieties (Red Creole, Chiltan-89, Local, Sariab, Surkh, White Globe and Local Kandhari) against thrips infestation in Quetta, Pakistan. The results revealed that local Kandhari followed by Sariab Surkh were most susceptible to thrips infestation while Chiltan-89 was the least. ALIMOUSI *et al.* (2007) have screened 15 Iranian onion genotypes against thrips incidence. The lowest thrips infestation (9.5-24.6), the percentage leaf infestation, leaf wax (0.0020- 0.0072 100 mg/g)

were seen in “Meshkan”, “Sefid-e-Kurdistan”, “Sefid-e-Qom” and “Eghlid” compared to susceptible genotypes.

Of 49 onion cultivars evaluated for resistance to thrips population and leaf damage, Colorado 6 and NMSU 03-52-1 had lowest numbers of thrips and leaf damage (JOHN *et al*., 2010). The varieties which were having glossy type of foliage showed comparatively lesser number of thrips as well as slight injury as compared to non glossy type. Because of glossiness thrips failed to lacerate the leaf surface and suck the sap whereas in non glossy leaves thrips can lacerate with its mandible and suck the sap. This may be due to presence any one of resistance mechanism such as non preference or antibiosis which comes in the way of thrips population build-up. Present findings are in accordance with MOGHADDAM *et al.* (2004) who observed that resistant onion cultivars had glossy foliage than the susceptible ones. Typical thrips injury was most prominent in genotypes with dark green non glossy foliage and was apparently absent in glossy foliage genotypes. The variety which recorded higher number of thrips was also recoded higher leaf thickness and there exists significant positive correlation between leaf thickness at bottom, middle and top with thrips population. However, in case of leaf thickness with press only, the leaf bottom thickness was positively correlated with the thrips population. The leaf thickness in case of onion may not be due to any hard tissue which prevents the insect to insert its stylet. In fact soft thickness might have facilitated the thrips to attack more and hence thicker leaf sheath ensured higher thrips population.

PAINTER (1951) reported that the differential thickness in wall of epidermal cells of cotton leaves might be of importance in imparting resistance to thrips attack. Thick cortex in the case of wild tomato relative of *Lycopersicon hirsutum* (L.) prevented the potato aphid, *Macrosiphum euphorbiae* Thomas from reaching the vascular bundles (QUIRAS *et al.,* 1977). This may be due to difference in the leaf structure and attacking insect. However, BORAH (1987) noticed that the intensity of thrips and mite attack did not exhibit any type of correlation with leaf thickness. The present study is in partial agreement with the finding. The least insect population and highest bulb yield resulted in to maximum net profit in AFLR treatment, which was followed by Bheema Super and the local cultivars of Bargarh has also recorded good yield which again proves the importance of local genotypes in well performance because of well plant acclimatization. Thus it can be concluded from the present investigation that varieties like Agrifound Light Red and Bheema Super are recommended for the region where as varieties like Red Ball and Nasik Red are found to be poor performers than the existing local variety.

REFERENCES

ALIMOUSAVI, S. A., HASSANDOKHT, M. R., AND MOHARRAMIPOUR, 2007. Evaluation of Iranian onion germplasms for resistance to thrips. *Int. J. Agri. Biol.,* **6**: 897-900

BORAH, D. C., 1987, Bioecology of *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and *Scirtothrips dorsalis* Hood (Thysanoptera : Thripidae) infesting chilli and their natural enemies. *Ph. D. Thesis, Univ. Agric. Sci., Dharwad, Karnataka (India).*

BRAR, K. S., SIDHU, A. S., AND CHADHA, M. L., 1993. Screening onion varieties for resistance to*Thrips tabaci* (Lind.) and *Helicoverpa armigera* (Hubner). *J. Insect Sci.,* **6**(1): 123-124.

BHARGAVA, K. K., AND ASHOK, B., 2005. Field evaluation of conventional and eco-friendlyinsecticides against onion thrips, *Thrips tabaci* L. (Thysanopetra: Thripidae). *J. Horti. Sci*., **11**(2): 74-79.

RAHMAN, K. A, BATRA, A. L. 1945. The onion thrips *Thrips tabaci* L. (Thripidae: Thysanoptera). *Indian Journal of Agricultural Sciences*, **19**(4): 308-310. 11

DIAZ-MONTANO, J., FUCHS, M., NAULT, B. A., AND SHELTON, A. M., 2010. Evaluation of onion cultivars for resistance to onion thrips (Thysanoptera : Thripidae) and iris yellowspot virus. *J. Econ. Entomol.* **103**: 925-937.

FOURNIER, F., BOIVIN, G., AND STEWART, R. K., 1995. Effect of *Thrips tabaci* (Thysanoptera: Thripidae) on yellow onion yields and economic thresholds for its management. *J. Econ. Entomol.* **88**: 140.

GOMEZ, K. A. AND GOMEZ , A. A. 1984. Statistical procedure for Agricultural research, *John Wiley and Sons*, 1-645.

JOHN, D. M., MARC, F., BRIAN, A. NAULT, AND SHELTON, A. M., 2010. Evaluation of onion cultivars for resistance to onion thrips (Thysanoptera: Thripidae) and Iris Yellow Spot Virus. *J. Econ. Entomol.,* **103**: 925 -937.

KENDALL, D. M., AND CAPINERA, J. L., 1990. Geographic and temporal variation in the sex ratio of onion thrips. Southwest. *Entomol*. **15**: 80- 88.

KENNEDY, G. G., 2003, Tomato, pests, parasitoids and predators: Tritrophic interactions involving the genus, *Lycopersicon*. *Ann. Rev. Entomol*., **48**: 51-72

MOGHADDAM, N. R., JALIANI, N., HABIBI, J. AND ALIAKBAR, A., 2004. Study of resistant sources of Iranian onion land races to thrips. *In : Proc.* 16th *Iranian Plant Prot. Cong., Aug-Sept., University of Tabriz, Tabriz, Iran*, p. 603

MALIK, M. F., NAWAZ, M., AND HAFEEZ, Z., 2003. Evaluation of promising onion (*Allium cepa*) varieties against thrips infestation in the Agro-ecosystem of Balochistan, Pakistan. *Asian J. Pl. Sci.,* **2**: 716-718

PAINTER, R. H., 1951. *Insect Resistance in Crop Plants,* The Macmillan Co., New York, p. 520.

PANDA, N., AND KHUSH, G. S., 1995, *Host Plant Resistance to Insects,* CAB International,Wallingford, United Kingdom, pp.10-12

PATIL, J. D., DESALE, G. Y., AND KALE, P. N. 1988. Storage studies in onion. *J. Maharashtra Agric. Univ.,* **12**: 114-115

PAWAR, D. E., MOTE, U. N., KALE, P. N., AND AJRI, D. S. 1987. Identification of resistant sourcesfor thrips in onion. *Curr. Res. Rep.,* **3**(2): 115-117.

QUIRAS, C. F., STEVENS, M. A., RICKS, C. M., AND KOK-YOKOMI, M. K., 1977. Resistance in tomato to pink form of potato aphid (*Hacroc slphumeurphorbae,* Thomas). The role of anatomy, epidermal hairs and foliage composition. *J. American Sco.Hortic. Sci.,* **102**: 166-171.

RUEDA, A., BADENES, P. F. R., AND SHELTON, A. M., 2007, Developing economic thresholds for onion thrips in Honduras. *Crop Prot*. **26**: 1099 -1107.

SHELTON, A. M., ZHAO, J. Z., NAULT, B. A., PLATE, J., MUSSER, F. R. AND LARENTZAKI, E., 2006,Patterns of insecticide resistance in onion thrips (Thysanoptera : Thripdae) in onion fields in New York. *J. Econ. Entomol.,* **99**: 1798-1804

SINHA, A. K., SINHA, R. B. P., AND AJAY KUMAR, 1995, Reaction of onion cultivars to thrips,*Thrips tabaci*. *J. Appl. Biol.,* **3**(1/2): 104-105.

SINGH, D., RAMZAN, M., AND GILL, S. P. S., 1977. Field incidence of thrips on different onion cultivars at Ludhiana. *Punjab Hort. J.,* **17**(4): 159-160.

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PIONEER COMMUNITIES IN BIOFOULING SUCCESSION ON THE VALIYAZHEEKAL HARBOUR, KERALA, INDIA

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ABSTRACT: Analysis of pioneer community in the biofouling succession was conducted during the first time in Valiyazheekal harbour, Kerala, India on 10th January 2015, 24 hrs after the panel immersion in the bay. The distribution of pioneer community during the early stages of succession was dominated by five species of bacteria, *viz*., *Staphylococcus lentus, Sphingomonas paucimobilis, Rhizobium radiobacter, Pantoea* sp and *Areococcus urinae.* The total bacterial count in the sample was 52x10-3 cfu/ml. The bacteria involved in the first colonisation stage may prepare micro-condition for the attachment of other subsequent fouling organisms such as diatoms, larval forms and foramniferans in the Valiyazheekal harbour.

Key words: Pioneer community, bacteria, succession, biofouling

INTRODUCTION

Microfouling is an event in the biofouling process. When an artificial substrate is first immersed into the marine environment, a series of changes occur on the surface of the substrate by marine organisms termed as **biofouling**. Primarily bacteria are the first organisms appear on the bare surface and multiply rapidly. The extra polymeric substrance (EPS) produced by bacterial colony attract diatoms, larvalforms etc. Biofilm is an assemblage of organisms like aggregates or micro-colonies of bacteria, protozoans, diatorms and invertebrate larvae (STOODLEY *et al.*, 2002). The primary biofilms are important for the recruitment of algal spores, diatoms and larval forms (WANG *et al.*, 2012; DOBRETSOV *et al.*, 2009). With in less than 30 minutes bacteria settle on the surface and begin to produce colonies and their population density attains peak within 24 hours. Marine bacteria are seen as rods, cocci, chains or they congregate into clumps or groups. 90% of the marine bacteria are Gram negative.

The adherence and the subsequent colonization of bacteria is the first stage in the development of biofouling succession on immersed hard substrata. The bacterial adhesion involves 2 stage processes (ROBB, 1984). Bacterial adhesion is due to physical force (weak London /van der Waals force) and electrostatic force of attraction. The attachment involves the attraction of bacteria towards a surface by the following process (1) reversible adhesion - the non-motile bacteria exhibit Brownian movement can be washed off by water (2) irreversible adhesion- the firm adhesion of bacteria cannot be removed by water current. Colony formation is the final stage, the cell multiply and increases its number to form micro colony which leads to the formation of initial bacterial film followed by the attachment of diatoms and other biofoulers. The early colonizers, the bacteria can influence the settlement of subsequent settlers through modification (physical and chemical) of the surface (SRIVASTAVA *et al.*, 1990). Bacteria in the biofilms control their growth by quorum sensing. Review focuses on the role of quorum sensing signalling and inhibition in marine bacteria by compounds derived from marine organisms and the formation of biofilm is considered to be an initial step in the development of marine biofouling (DOBRETSOV *et al.*, 2009).

Most of the studies revealed that the ∝-proteobacteria was the dominant member in marine biofilms (SALTA *et al.,* 2013). γ-proteobacteria and ∝-proteobacteria have been identified as the primary colonizers on hard substrates submerged in marine waters (CHUNG *et al.*, 2010; DOBRETSOV *et al.*, 2013) Studies on microfouling development on artificial substrate (Nylon nets) deployed in Central Red Sea showed after 24 hr bacteria and diatoms being the primary colonizers (AHMED *et al.*, 2018). The substrate surface properties are altered by the conditioning film that enables the attachment of microorganisms especially bacteria (BHOSLE *et al.*, 2005). LEE *et al.* (2008) reported that the early stage of biofilms was dominated by the bacteria that were most abundant in planktonic communities. Role of microbial film in biofouling was studied in India by DANIEL (1955), MEENAKUMARI and NAIR (1994). NAIR AND RAVINDRAN (1994) studied the dynamics of bacterial colonization and barnacle settlement on the surface treated with repellants and attractants.

Biofilms play a key role in primary production, biodegradation of organic matter, environmental pollutants and nutrient recycling; the primary colonizers provide specialized nutrients for the secondary colonizers (DECHO, 2000; THOMPSON *et al.,* 2004; PASSARELLI *et al.,* 2015). The modification of a surface by the initial colonizing bacteria may render it more suitable for colonization by other groups of biofoulers. Most of the marine bacteria are heterotrophic and they perpetuate by mineralising organic matter and survive under a variety of changing condition. They have the ability to perform photosynthesis by using bacteriochlorophyll, nitrification convert molecular nitrogen, ammonia and oxides of nitrogen into nitrate, dentitrification (FAN *et al.*, 2015). Phosphorus is also generated by bacterial activity when compared to terrestrial bacteria; they multiply rapidly by fission without spores. The hydrological parameters, the temporal and spatial variation in their distribution also affect the recruitment and the settlement pioneers and other subsequent colonizers. The main objective of the present study is to analyse the pioneer community attached in the panels retrieved after 24 hours during biofouling succession studies that will result in the colonization of other biofoulers on Valiyazheekal, a fishing harbour on the South West coast of India.

MATERIALS AND METHODS

Valiyazheekal harbour ($9^0$2'9''N latitude to $76^0$25'32''E longitude) is a small fishing harbour that lies 6 km west of Kayamkulam town in Alappuzha district, Kerala is an ancient maritime trading centre. Kayamkulam kayal opens in to the Lakshadweep Sea through Valiyazeekal Azhi, a permanent opening where the fishing vessels enter in to the sea and return to the harbour. Pulimuttu is constructed in the harbour area characterised by manmade rocky shore directly facing the sea exposed to waves, provide extra habitat for biofoulers and their succession. For the analysis of pioneer community in the biofouling succession on Valiyazheekal harbour, artificial coupons (10x5cm) were immersed in the bay. The coupons include panels of cement; PVC, glass, wood, and iron were deployed 1m depth in the bay on 9th January 2015. After 24h the panels were retrieved and the samples were taken by gently swabbed on the surface of the panels. These swabs were given for detailed analysis of pioneers settled on the test panels. Analysis was done at CEPCI (Cashew Export and Promotion Council of India Laboratory and Research Institute), Kollam, Kerala.

The total count of bacteria in the sample was done by using pour plate technique and the reference method was IS 1622-1981, Edition 2.4 (2003-05) IS 5402:2002/ISO 4833:199). After bacterial enumeration, colonies were selected (isolated from plates) showing different morphology and the colonies were subjected to bacterial identification. The identification was done by phenotypic automation equipment VITEK 2 (Version 7.01) compact Biomerieux, France. Bacterial colonies isolated were purified in

non selective agar PCA (Plate Count Agar) and gram staining was done. Based on the gram staining reaction VITEK cards were selected for gram positive and gram negative bacteria. Twenty four hour colony was taken and 3 ml normal saline was taken and inoculums turbidity was adjusted to require level for each group of bacteria. After adjusting required turbidity cards filler was inserted cuvettee containing normal saline and inserted in the filler portion of the machine for filling the sample inoculums in the cards. After 30 seconds the filled cards were taken out and inserted in the reader machine for biochemical identification. An average time taken to complete the identification varies depending on the organisms. About 5 to 8 hours will take to complete the analysis (GN cards for gram negative fermenting and non-fermenting bacilli and GP cards for gram positive cocci and non spore forming bacilli). To confirm the bacterial species identity based on Vitek cards, automated computer based method of species identification via the measurement of light attenuation associated with each biochemical reaction (SHETTY *et al.*, 1998). This technique employs over 20 different biochemical tests in reaction to assess the growth and viability of bacterial strains (JANDA AND ABBOT, 2002).

RESULTS AND DISCUSSION

The results of the analysis of 24 hour samples taken from the test panels after biochemical test (Fig.1) showed that the pioneer community (Fig.2) consists of five species of bacteria comprising *Staphylococcus lentus* with 97% probability *Sphingomonas paucimobilis* (94%), *Rhizobium radiobacter* (99%), *Pantoea* sp (85%) and *Areococcus urinae* (90%). The total bacterial count on the sample is $52x10^{-3}$ cfu/ml (cfu - colony forming units).The identification details of the pioneer community settled on panels during study are as follows:

1. *Staphylococcus lentus*

Kingdom - Bacteria
Phylum - Firmicutes
Class - Bacilli
Order - Bacillales
Family - Staphylococcaceae
Genus - ***Staphylococcus***
Species - ***lentus***

Biochemical details

2	AMY	+	4	PIPLC	-	5	dXYL	+	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	+	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TryA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	+	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

S. lentus is a gram positive, salt tolerant, oxidase positive, coagulase negative clustered cocci. The contraindicating typical biopattern is NOVO (20). They exhibit facultative metabolism. Macroscopic structure of most colonies appears relatively smooth, glossy, butyrous, and sometimes appearing wet. Colonies of most strains are usually opaque and may be pigmented white or cream and sometimes yellow to orange.

2. *Sphingomonas paucimobilis*

Kingdom - Bacteria

Phylum - Proteobacteria

Class - Alphaproteobacteria

Order - Sphingomonadales

Family - Sphingomonadaceae

Genus - ***Sphingomonas***

Species - ***paucimobilis***

Biochemical details

2	APPA	+	3	ADO	-	4	PyrA	+	5	IRAL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	LATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	D129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

S. paucimobilis is an aerobic oxidase positive, catalase positive, motile gram negative, rod shaped, and chemoheterotrophic bacteria. They possess glycosphingolipids in their cell envelop and produce yellow pigmented colonies. The contraindicating typical biopatterns are PyrA(24), PLE(24), dMAN(20). They can survive in low nutrient concentration and can metabolize variety of carbon sources. The diversity of the family Sphingomonadaceae was higher in the young biofilms than in the old biofilms. It has been suggested that members of the Sphingomonadaceae are pioneers in biofilm formation (ZHANG *et al.*, 2006). Bacterial density is an important parameter in the induction of the larval settlement of *H. elegans* (HUANG AND HADFIELD, 2003).

3. *Rhizobium radiobacter*

Kingdom - Bacteria

Phylum - Proteobacteria

Class - Alphaproteobacteria

Order - Rhizobiales

Family - Rhizobiacea

Genus - ***Rhizobium***

Species - ***radiobacter***

Biochemical details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IRAL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	LATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

R. radiobacter is a saprophytic, gram negative rods belonging to alpha-proteobacteria and was a dominant member of microbial community in sediment depths (BATZKE *et al.*, 2007).

4. *Pantoea* sp.

. Kingdom - Bacteria
Phylum - Proteobacteria
Class - Gamma Proteobacteria
Order - Enterobacteriales
Family - Enterobacteriaceae
Genus - ***Pantoea* sp.**

Biochemical details

2	APPA	+	3	ADO	-	4	PyrA	-	5	IRAL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	+	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

Pantoea sp. is a gram negative motile bacillus shaped aerobic bacteria in the family of Enterobacteriaceae. The contraindicating typical biopatterns of *Pantoea sp.* are GGAA(1), APPA(1), AGLU(1). They are yellow pigmented, form mucoid colonies and ferment lactose. Some species show quorum sensing ability that could drive different gene expression, hence controlling certain physiological activities.

5. *Areococcus urinae*

Kingdom - Bacteria
Phylum - Firmicutes
Class - Bacilli
Order - Lactobacillus
Family - Aerococcaceae
Genus - ***Areococcus***
Species - ***urinae***

Biochemical details

2	AMY	-	4	PIPLC	-	5	dXYL	+	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TryA	+	30	dSOR	+	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-
47	NOVO	+	50	NC6.5	-	52	dMAN	+	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	-															

A. urinae is a gram positive cocci, occur mostly in clusters, micro aerophilic, catalase negative and found in wide range of environments including marine sources. The typical contraindicating biopatterns are ADH1(1), dXYL(1). The induction of pioneer community (bacterial colonies) favours the settlement and colonization of diatoms, larval forms and Foramniferans in the test panels. Diatoms include *Navicula* sp*., Nitzschia* sp*., Conscinodiscus* sp*.* and *Synedra* sp*.* are common and settled in high rates in test panels of wood, cement, glass, PVC and iron. Foramniferans such as *Golbigerina* sp*.* and *Elphidium sp.* settled in large numbers. The nereid larval forms are abundant in all the panels. Hence it is clear that the pioneer community of bacteria favours the settlement of the secondary colonizers. The opportunistic pioneer species are weak competitors with high growth rate and they quickly monopolize the resources. The open space i.e, the substrate confers advantages to the primary colonizers because they can exploit open substrata for short period (PAINE, 1977).

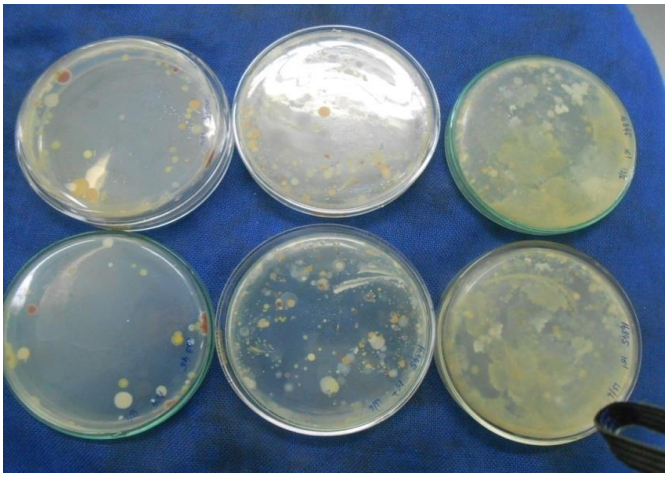

Fig. 1 Bacterial culture for the isolation of pioneer community

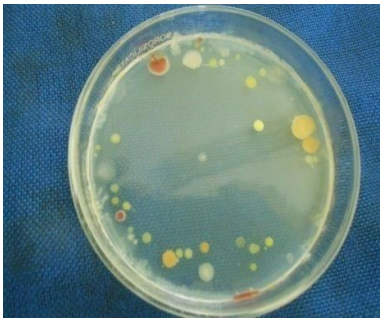

Fig. 2 Pioneer community of bacteria isolated from the panel

Marine bacterial biofilms are important for the settlement of planktonic larvae of marine invertebrates (CRISP, 1974). The initial colonizers are predominantly chemo organotrophic, gram negative rods. The colonization of hard surface by bacteria is a stimulus for settling of the fouling larvae (MITCHELL and KIRCHMAN, 1984). Larval forms of spirorbid polychaetes, bryozoans species and oysters respond positively to bacterial biofilms on the surface they attach (WEINER *et al.*, 1985). Larvae of benthic forms of polycheates settle on sand colonized by bacteria and microorganisms (GRAY,

1966). During the present study the bacterial biofilms on the panels attract the larva of polycheates and barnacles i.e., secondary colonizers. Earlier studies have shown that biofilms developed on different artificial substrata such as concrete, fiberglass, glass, and cement, contain unique microbial communities that correlate with the differential settlement of barnacle larvae (FAIMALI *et al.*, 2004; HUNG *et al.*, 2008). The bacterial community structure has been suggested as an indicator of habitat suitability for settling (UNABIA and HADFIELD, 1999; QIAN *et al.*, 2003) and induces the settlement of larvae and metamorphosis of *Hydroides elegans* (LAU *et al.,* 2005, NEDVED and HADFIELD, 2009).

In **conclusion**, the present study envisages that pioneer community in the hard substrata deployed on Valiyazheekal Harbour consists of five species of bacteria comprising *Staphylococcus lentus* with 97% probability *Sphingomonas paucimobilis* (94%)*, Rhizobium radiobacter* (99%), *Pantoea* sp (85%) and *Areococcus urinae* (90%). The colonization of bacteria is the first stage in the development of biofouling succession on immersed hard substrata; bacterial colonies initiate the subsequent colonization of secondary and tertiary biofoulers in this harbour.

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REFERENCES

AHAMED, A. BALQADI, ADNAN, J. SAAMA, and SATHIAANESON, S. 2018. Microfouling development on artificial substrates deployed in the Central Red Sea, *Oceanologia*, **60** (2): 219-213.

BATZKE, A., ENGELEN, B., SASS, H., and CYPIONKA, H. 2007. Phylogenetic and physiological diversity of cultured deep-biosphere bacteria from equatorial Pacific Ocean and Peru Margin sediments. *Geomicrobiol J.* **24**: 261–273.

BHOSLE, N. B., GARG, A., FERNANDES L. and CITON, P. 2005. Dynamics of amino acids in the conditioning film developed on glass panels immersed in the surface seawaters of Dona Paula Bay. *Biofouling,* **21**(2): 99-107.

CHUNG, H., LEE, O., HUANG, Y., MOK, S., KOLTER, R.and QIAN, P. 2010. Bacterial community succession and chemical profile of subtidal biofilms in relation to larval settlement of the polychaete Hydroides elegans *ISME J.* **4**: 817-828.

CRISP, D. J. 1974**.** Factors influencing the settlement oof marine invertebrate larvae. *In PT Grant and A Mackie, eds. Chemoreception in mmarine Academic Press, New York,* pp 177-265.

DANIEL, A.1955. The primary film as factor in the settlement of marine fouler. *J. Madras Univ*. **(B)52**: 189.

DECHO, A. W. 2000. Microbial biofilms in intertidal systems:an overview.*Cont. Shelf Res*. **20**(10): 1257-1273.

DOBRETSOV, S., TEPLITSKI, M. and PAUL, V. 2009. Minireview: qurom sensing in the marine environment and its relationship to biofouling. *Biofouling* **25**(5)*:* 413-427.

DOBRETSOV, S., ABED, R. M. M. and TEPLITSKI, M. 2013. Mini-review: inhibition of biofouling by marine microorganisms, *Biofouling,* **29**(4): 423-441.

FAIMALI, M., GARAVENTA, F., TERLIZZI, A., CHIANTORE, M. and CATTANEO-VIETTI, R. 2004. The interplay of substrate nature and biofilm formation in regulating *Balanus amphitrite* Darwin, 1854 larval settlement. *J. Exp. Mar. Biol. Ecol.* **306**: 37–50.

FAN, H., BOLHIUS, H., LUCAS and J. STAL, 2015.Nitrification and Nitrifying bacteria in coastal mud mats.*Front. Microbiol*. **6**: 1367

GRAY, J. S. 1966. The attractive factor of intertidal sands of Protodrilus symbioticus. *J. Mar. Biol. Assoc. UK,* **46**(3): 627.

HUANG, S. Y. and HADFIELD, M. G. 2003. Composition and density of bacterial biofilms determine larval settlement of the polychaete *Hydroides elegans*. *Mar. Ecol. Prog. Ser*. **260**: 161–172.

HUNG, O., THIYAGARAJAN, V. and QIAN, P. 2008. Prefrential attachment of barnacle larvae to natural multi-species biofilms: does surface wettablity matter? *J. Exp. Mar. Biol. Ecol.* **361**: 36 – 41.

JANDA, J. M. and ABBOT, S. L. 2002. Bacterial identification for publication: when is enough enough? *J. Clin. Microbiol.* **40**(6): 1887-1891.

LAU, S. C. K and Qian, P. Y. 2002. Larval Settlement in the serpulid polychaete. Hydroides elegans in response to bacterial film: an investigation of the nature of putative larval settlement cue. *Mar. Biol.* **138**(2): 321-328.

LAU, S. C. K., THIYAGARAJAN, V., CHEUNG, C. K. and QIAN, P.Y. 2005. Roles of bacterial community composition in biofilms as a mediator for larval settlement of three marine investebrates. *Aquat. Microb. Ecol.* **38**(1): 41-51.

LEE, J. W., NAM, J. H., KIM, Y. H., LEE, K. H. and LEE, D. H. 2008. Bacterial communities in the initial stage of marine biofilm forrmartion on artificial surface, *J. Microbiol.* **46**: 174-182.

MEENAKUMARI, B. and NAIR, N.B. 1994. The effect of slime film on the barnacle settlement. Recent Developments in Biofouling Control. Oxford *& IHB Publishing Co. Pvt. Ltd. New Delhi*, pp 3-10.

MITCHELL, R. and.KIRCHMAN, D 1984. The microbial eclogy of marine surface in J.D. Costlow and Tipper, eds. Marine Biodeterioration: An interdisciplinary study. *Naval Institute Press, Annapolis,* pp 49-56.

NAIR, N. B. and RAVINDRAN, K. 1994. Studies on chemosensor induced surface modificationson the control of biofouling dynamics. *Recent Developments in Biofouling Control. Oxford & IHB Publishing Co. Pvt. Ltd. New Delhi,* pp 313 - 320.

NEDVED, B. T. and HADFIELD, M. G. 2009. Hydroides elegans (Annelida: Polychaeta): A Model for Biofouling Research. *Marine and Industrial Biofouling*, *Springer Series on Biofilms*, pp 203 – 217.

PASSARELLI, C., MEZIANE, T., THINEY, N., BOEUF, D., JESUS, B., RUIVO, C., JEANTHON, C. and HUBAS, C. 2015.Seasonal variation of the composition of microbial biofilms in sandy tidal flats: focus of fatty acids pigments and exopolymers. *Estuar.Coast. Shelf. Sci.***153**: 29-37.

PIANE, R. T. 1977.Controlled manipulation in marine intertidal zone and their contributions to ecological theory. *The Changing Scenes in the Natural Sciences Academy of Natural Sciences, Philadelphia*.pp.245-270.

QIAN, P. Y., THIYAGARAJAN, V., LAU, S. C. K. and CHENG, S. C. K. 2003. Relationship between bacterial community profile in biofilm and attachment of the acorn barnacle *Balanus amphitrite*. *Aquat. Microbial. Ecol*. **33**: 225–237.

ROBB, I. D. 1984. Stereo- biochemistry and function of polymers. *In: Microbial Adhesion and Aggregation (Dahlem Konferenzen), K. C. Marshell (ed.), Springer-Verlag, Berlin*, pp.39-49.

SALTA, M., WHARTON, J. A., BLACHE, Y., STOKES, K. R. and BRIAND, J. F. 2013. Marine biofilms on artificial surfaces: structure and dynamics.*Environ. Microbiol*. **15** (11): 2879-2893.

SHETTY, N., HILL, G. and RIDGWAY, G. L. 1998. The Vitek Analysis for routine bacterial identiication and susceptibility testing: protocols, problems and pitfalls. *J. Clin. Pathol*. **51**(4): 316 - 323.

SRIVASTAVA, R. B., GAONKAR, S. N. and KARANDAE, A. A.1990. Biofilm characterization in coastal waters of Bombay, *Proc. Indian Acad. Sci. Animal Science* **99**:163-173.

STOODLEY, P., SAUER, K., DAVIES, D. G. and COSTERN, J. W. 2002. Biofilms are copplex differentiated communities. *Annual Review of Microbiology* pp 187-209.

THOMPSON, R. C., NORTON, T. A., HAWKINS, S. J., 2004. Physical stress and biological control regulate the producer-consumer balance in intertidal biofilms.*Ecology* **85**(5): 1372-1382.

UNABIA, C. R. C. and HADFIELD, M. G. 1999. Role of bacteria in larval settlement and metamorphosis of the polychaete *Hydroides elegans*. Mar. Biol. **133**: 55–64.

WANG, C., BAO, W. Y., GU, Z. Q., LI, Y. F., LIANG, X., LING, Y., CAI, S. L., SHEN, H. D. and YANG, J. L. 2012. Larval settlement and metamorphosis of the mussel *Mytilus coruscus* in response to natural biofilms. *Biofouling*, **28** (3)*:* 249-256.

WEINER, R. M., SEGALL, A. M. and COLWELL, R. R. 1985. Characterization of a marine bacterium associated with *Cassostrea virginica* (the Eastern Oyster) *Appl. Environ. Microbiol*. **49**:83-90.

ZHANG, K., CHOI, H., DIONYSIOU, D. D., SORIAL, G. A. and OERTHER, D. B. 2006. Identifying pioneer bacterial species responsible for biofouling membrane bioreactors. *Environ. Microbiol.* **8**: 433–440.

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FEEDING POTENTIAL OF COCCINELLID PREDATOR, *MENOCHILUS SEXMACULATUS* (FABRICIUS) (COLEOPTERA: COCCINELLIDAE) ON COWPEA APHID, *APHIS CRACCIVORA* (KOCH)

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ABSTRACT: The laboratory studies conducted on the feeding potential of the coccinellid predator, *Menochilus sexmaculatus* (Fabr.) on cowpea aphid, *Aphis craccivora* (Koch) revealed that the neonate grubs just after hatching started feeding on chorion of the eggs, and then fed on the aphids. The feeding potential increased consequently from 1st, 2nd, 3rd, and 4th instar by consuming an average number of 23.5±1.24, 32.5±0.38, 38.75±0.90 and 61.37±0.74 aphids/day, respectively. Maximum number of aphids was consumed by adult of 93.33±0.03 aphids/day.

Key words: *Menochilus sexmaculatus*, feeding potential, *Aphis craccivora*

INTRODUCTION

Aphids are tiny, soft bodied, sucking pests and approximately 4000 species of aphids have been observed so far feeding over 250 agricultural and horticultural crops. These cause damage directly by sucking cell sap, secrete honey dew on leaves and shoots, and indirectly as vector of plant viruses (RABOUDI *et al*., 2002). On several occasions, insecticidal applications have accentuated the aphid populations and quite often resulted in outbreaks. However, predatory coccinellids play a major role in keeping these under control (KRISTOPHER *et al.,* 2002).

Among the predatory coccinellids, *Menochilus sexmaculatus* (Fabricius), is one of the potential predators of aphids and is widely distributed in India, Iran, Australia and other parts of the Oriental region (AGARWALA and BARDHANROY, 1997). The important features of *M. sexmaculatus* includes its wide geographic distribution and host range, broad habitats, tolerance to certain pesticides, enhanced searching ability, voracious larval feeding capacity and easy rearing in laboratory (VENKATESAN *et al.,* 2006). The feeding potential of coccinellids varies with their food and changes with the environmental conditions. Therefore, the present study was undertaken to determine the predatory efficiency of grubs and adults of *M. sexmaculatus* on cowpea aphid host, *Aphis craccivora* Koch.

MATERIAL AND METHODS

The present studies were conducted in the Instructional farm and Bio control laboratory, department of Entomology of IGKV, Raipur, during 2017-18. The cowpea aphids were collected from experimental farm of IGKV and adults of *M. sexmaculatus* were collected from pigeon pea, brinjal and cowpea crops from IGKV campus. These were transferred to glass jar (22.5 x 15 cm) and they were used as a nucleus culture for mass multiplication. The bottom of the jar was covered with Whatman no. 1 filter paper. Cowpea twigs infested with *A. craccivora* were given as food for adult beetles. Oviposition took place inside the jar. Egg masses were also laid on cowpea twigs which were removed gently and kept for further development (GAUTAM, 2008). The cultures were maintained under laboratory conditions at 27±10 ^{0}C and 60±5% RH. Active and healthy grubs and adults were selected for further experimental purpose. Freshly

collected aphid hosts, *A. craccivora*, were placed on fresh and tender cowpea, which were then placed over the filter paper. Single grub was released to each petri-plate (6x 1 cm) with the help of camel hair brush. Aphids were supplied every morning at the rate of 40, 60, 80 and 100 in number for 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instars, respectively. The number of aphids consumed within 24 hr. and duration of each instar were recorded. To determine predation efficiency of adult, newly emerged adults were transferred individually in petri-plates and aphids were supplied every day at the rate of 100 numbers for an adult on respective leaf and twigs. The number of aphids consumed by an adult beetle was recorded at 24 hr. intervals.

Eggs laid on the cowpea twigs, and tissue papers placed in the rearing jars were gently separated and kept in small petri-plate (6 x 1cm) hatching. The period between egg laying and hatching was considered as incubation period. Newly hatched grubs were kept individually in small plastic petri-plates (5 × 1.5 cm) and provided with sufficient food throughout their developmental period. Observations were taken at 12 hr. intervals to record the duration of each instar, the summation of each of which gave the total larval period. Experiment was replicated four times (CHANDRABABU *et al.* 1999). The data were subjected to square root transformation and statistically analyzed with CRD using OPSTAT.

RESULTS AND DISCUSSION

The predatory efficiency of the grub was determined by counting total number of aphids consumed by each larval instar. The consumption rate increased gradually from the first to fourth instar (Table-1). Consumption of first instar as (23.5±1.24 aphids/day) by the test predator was recorded while predation of 2^{nd} instar grubs of (32.5±0.38 aphids). Third instar grub showed better predatory activity than first two instars and the fourth and final instar grubs were very active until pupal transformation.

Table-1: Feeding efficiency of *M. sexmaculatus* on cowpea aphid (*Aphis craccivora*)

	R1	**R2**	**R3**	**R4**	**Mean**
1st instar	21.00	19.00	30.00	24.00	23.5±1.24 (4.93)
2nd instar	32.00	31.00	34.00	33.00	32.5±0.38 (5.79)
3rd instar	43.00	35.00	38.00	39.00	38.75±0.90 (6.30)
4th instar	57.5	62.00	63.5	62.5	61.375±0.74 (7.90)
Adult	92.21	92.29	92.35	92.46	92.33±0.03 (9.66)
C.D.					0.394
SE(m)					0.13

*Figures in parentheses are square root transformation values

They required more food than the previous instar due to bigger size, longer duration and to accumulate nutrient for pupal period. In the 3^{rd} instar, the mean consumption was estimated to be 38.75**±**0.90 aphids/day. During 4^{th} instar, they devoured a maximum number of 61.37**±**0.74 aphids/day. Duration of all the four instars were found to be of one day in the present studies, however, the adult stage prolonged for about 25-43 days. It was found that the female consumed more prey than the male and the longevity of adult beetle ranged from 25-43 days.

Thus the studies conducted on the feeding potential of coccinellid predator, *M. sexmaculatus* on cowpea aphid, *A. craccivora* revealed that the mean maximum number

of aphids consumed increased from 1st to 4th instar with maximum number of aphids were consumed by adult *i.e.,*93.33±0.03/day. Feeding potential of *M. sexmaculatus* grubs increased consequently from 1st, 2nd, 3rdand 4th instar by consuming an average number of 23.5±1.24, 32.5±0.38, 38.75±0.90 and 61.37±0.74 aphids/day, respectively. This is the first report on predation potential of *M. sexmaculatus* on aphid, *Aphis craccivora* at Raipur in the state of Chhatigarh in India.

REFERENCES

AGARWALA, B. K. and BARDHANROY, P. 1997. Oviposition behaviour and reproduction efficiency in ladybird beetles (Coccinellidae: Coleoptera): A case study of *Menochilus sexmaculatus* (Fabricius). *Journal of Aphidology,* **11**: 49-59.

CHANDRABABU, A., GAUTAM, R. D. and GARG, A. K. 1999. Biology of the ladybird beetle *Brumoides suturalis* (Fabr.) on aphid and mealy bugs. *Annals of Pl. Prot. Sci.***7**(1): 13-18.

GAUTAM,R.D. 2008. Mass rearing techniques of laboratory hosts and predators. *Biological pest suppression*, *Westville Publishing House, New Delhi*. 80-123pp.

KRISTOPHER, L., GILES, I., RICHARD, C., BERBERET., ALI, A. Z. and JACK, W. D. 2002. Influence of alfalfa cultivar on suitability of *Acyrthosiphon kondoi* (Homoptera: Aphididae) for survival and development of *Hippodamia convergens* and *Coccinella septempunctata* (Coleoptera: Coccinellidae). *J. Econ. Entomol.,* **95**: 552-557.

Raboudi, F., Ben Moussa, A., Makni, H., Marrakchi, M. and Makni, M. 2002. Serological detection of plant viruses in their aphid vectors and host plants in Tunisia. *Environmental Plant Protection Organization Bulletin,* **32**: 495-498.

Venkatesan, T., Jalali, S. K., Murthy, K.S. and Bhaskaran, T. 2006. Rearing of *Cheilomenes sexmaculata* (Fabricius) on artificial diet and its predatory efficiency against *Aphis craccivora* Koch. *Annals of Plant Protection Sciences,* **14**(2): 277-279.

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GREEN SYNTHESIS OF GOLD NANOPARTICLES AND THEIR ASSESSMENT ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN FISH, *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT: A green approach to synthesize gold nanoparticles using *Curcuma longa* extract is described at room temperature. The obtained gold nanoparticles were characterized by UV-Vis spectroscopy. The implications of colloidal AuNPs supplementation on haematological and biochemical functions of freshwater fish, *Oreochromis mossambicus* were evaluated. Fishes were feeding for 60 days with control diet and experimental diet containing green gold nanoparticle, respectively. The present results demonstrated dietary supplementation of gold nanoparticle which showed significantly increased haematological and growth indices in *O. mossambicus* with control and other experimental group throughout the experimental period. The results prove that the inclusion of green gold nanoparticle enhances growth, and elicits immunity to a significant level. Therefore, the plant mediated synthesized colloidal AuNPs can be used as an "immunomodulator" in aquaculture sectors instead of synthetic growth factors.

Key words: Gold nanoparticles, Immunomodulator, *Curcuma longa*, green gold

INTRODUCTION

During the last few decades, metal nanoparticles have elicit much interest due to their distinct physical, chemical and biological properties and had become most active area of research during past few decades (HUBENTHAL, 2010). Owing to the interest and importance of nanoparticles many researchers have focused on the synthesis of nanoparticles using various chemical and physical methods. These methods available for the synthesis of gold nanoparticles like ion sputtering, reverse micelle, chemical reduction, hydrothermal, sol gel, etc. but unfortunately, are quite expensive and potentially hazardous to the environment which involve use of toxic and perilous chemicals that are responsible for various biological risks (YU, 2007). The technique using naturally occurring reagents such as plant extracts, fungi, sugars, and bacteria, biodegradable polymers as reluctant and stabilising agents could be considered alternative for synthesis of inorganic nanoparticles. The synthesis of nanoparticles using plant extract provides advancement over other methods as it is simple, one step, cost-effective, environment friendly and relatively reproducible (ABHIJITH and THAKUR 2012).

In our approach to green synthesis, we selected turmeric rhizomes to produce gold nanoparticles. The plant *Curcuma longa* L. belongs to *Zingiberaceae* family. This rhizome has been used for many medical applications such as treatment of burns, hot swellings, small pox, ulcers of the mouth and stomach (KHANNA, 1999). It is also used as an anti-irritant and anti-microbial. The antioxidant property of curcumin can prevent rancidity of food and thus provides foodstuff with less oxidized fat and free radicals. Keeping the biological perspective in mind, in the present study, we demonstrate the use of turmeric rhizome extract for the synthesis of gold nanoparticles. Diseases out breaks are particularly prevalent in rapidly developing aquaculture industries, affecting the

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economic development of this sector. The uses of antibiotics as a growth promoter in basal diets are in practice (CROMWELL, 1991). However, continuous use of antibiotics leads to its retention in animal tissues, which may provoke antibiotic resistance both in animals and consumers thereby raising questions for food security. There is a need for ecofriendly disease prevention measures to promote suitable culture. The most effective method may be the development of natural diseases resistance in fish; with immunostimulants which can increase the immunopotency and disease resistance of fish.

In this scenario, dietary inclusion of trace elements and nanoparticles is an ideal option. Gold -based compounds have been used in many bactericidal applications. Nanogold is a submicronic and colloidal form of metallic gold (1-100 nm size) which has presently got wide commercial attention due to its pronounced impact than bulk gold metal as antimicrobials. One of our recent studies shows gold nanoparticles is more effective against Gram-positive and Gram-negative pathogens (DHANYARAJ *et al.,* 2017). There is very limited information on dietary application of gold nanoparticles in fish feed. Therefore, this is a timely approach to evaluate the encouraging effects of dietary nanoparticles within nontoxic levels of administration. In the present study, we tried to monitor the effects of biosynthesized AuNPs, on modulation of hemotological and biochemical parameters in freshwater fish, *O.mossambicus.*

MATERIALS AND METHODS

Chemicals, plant and experimental fishes: Hydrogen tetra choloroaureate (III) hydrate $HAuCl_4.3H_2O$ (99.9%) was purchased from Sigma–Aldrich, Bangalore. All other chemicals and solvents used in this study were of analytical grade and obtained from Merck, Mumbai, India. *Curcuma longa* was purchased from Pankajakastoori Ayurvedic Research Centre Trivandrum. *Oreochromis mossambicus* were collected from Agency for Development of Aquaculture in Kerala (ADAK) at Varkala (2018 February).

Preparation of plant extract: The rhizome, of *Curcuma longa* was washed thoroughly with tap water to get rid of debris. About 1 g of *C. longa* was mixed with 10 mL of distilled water and crushed in a mortar pestle. The aqueous extract of *C .longa* was filtered with Whatman No. 4 filter paper. The filtered extract was centrifuged at 1000 r/min for 10 min. The supernatant was collected and was kept at room temperature.

Green synthesis of GNPs: Preparation of gold nanoparticle was done according to the method described by SREE LEKSHMI *et al*; 2013 with slight modifications. Biogenic gold nanoparticle was synthesized using Tetra Chloro auric acid solution ($HAuCl_4.3H_2O$ and *C. longa* extract. In a conical flask, 10 ml of *C. longa* extract was added to10µl of aqueous solution of 0.3M Chloroauric acid solution ($HAuCl_4.3H_2O$) at room temperature under static conditions. The reduction of Au NPs was clearly observed within the next 5-30 min. The solution has been modified from pale yellow to pink colour, which indicates the formation of biogenic (Au/*C. longa)* NPs.

Characterization of biosynthesized AuNPs

The reduction of Au-NPs was confirmed by using UV-vis spectroscopy in the range of 500 to 600nm (Shimadzu, UV-1601 UV-VIS Spectrometer). In this study, fresh and healthy fishes, with initial body weights of 26.65±0.238 gm were used. At the end of acclimatization, the fishes were transferred to aquarium tanks that maintained the conditions identical to the stock tanks. Water quality parameters were maintained everyday and monitored periodically and proper aeration was provided during the whole experimental period. 40-50% of water was removed every day to remove faecal materials and unused feed was conducted in glass aquaria and kept for ten days for stabilization.

Three treatment combinations were made as: TD1- Control, TD2 - diet with *C.longa* plant extract, TD3- diet with biogenic gold nanoparticles with eight fish in each. Water quality parameters were monitored. The physico-chemical parameters of water such as temperature (28±2°C), dissolved oxygen (5.8±0.30mg/l), pH (7.497±.0.19) and total alkalinity (198.3±4.3mg/l) were in optimum range during the experimental period. Final body weights of fishes were taken after the 60 days of rearing.

Three isoproteinous diets were prepared by mixing rice bran (13.24%), ground nut oil cake (36.76%), tapioca flour (13.24%) and fish meal (36.70%) (Hardy R 1980). The experiment feed proximate composition were represented in Table 1 (JOHNSON, 2004).The control treatment fishes were fed with normal diet (TD1), while experimental fishes were fed with diet having (TD2 , TD3@ 4% body weight per day in two equal instalments for 60days.

- TD1[Control diet]
- TD2[10ml *C.longa* extract in 100gm basal feed]
- TD3[10µl($HAuCl_4.3H_2O$+10ml *C.longa* extract in 100gm basal feed

Table-1: Proximate composition of basal feed

Properties (%)	**Composition ± SD**
Moisture	10.16±0.17
Protein	40.41±0.08
Lipid	9.18±0.18
Carbohydrate	20.01±0.03
Fiber	4.81 ± 0.14
Ash	12.98±0.18

Haematological parameters: At the end of the experiment, fish were fasted overnight and then blood samples were drawn from randomly collected fish, through the caudal vein. The blood samples were used for determining erythrocyte count, white blood count and haemoglobin content (JOHNSON, 2002). The plasma was obtained by centrifugation of blood at 3000 r/min for 15 min and non haemolysed plasma was stored in deep freezer for further biochemical analysis. Serum protein was estimated by Lowry method (LOWRY *et al.,* 1951). Albumin was estimated by using albumin kit (Span diagnostic LTD India).Globulin was calculated by subtracting albumin values from total protein. The values were expressed as mean ± SD. Statistical analysis was performed by one way analysis of variance (ANOVA). The significant difference among means was determined by Duncan's multiple range test (Duncan, 1955) at the level, $p<0.05$ were considered as significant**.**

RESULTS AND DISCUSSION

By using CL (*Curcuma longa*) extract, we fabricated GNPs at room temperature, and the formations of GNPs were observed by visual color change and conformed by UV-visible spectroscopy. The color change observed from light yellow to pink within 15 min after the addition of plant extract to aqueous HAuCl*4*, which is the characteristic for the formation of CL -GNPs due to the excitation of surface plasmon vibrations of Au+ ions. It is well known that the optical properties of the metal nanoparticles are strongly dependent on their size and shape. The absorption spectrum of the C.L aqueous extract has shown in Fig. 1(a) exhibits a characteristic band at 410nm. The disappearance of the UV-Vis absorption band at 410 nm and appearance of a new band at 540 nm could be attributed

to the formation of AuNPs. The UV-Vis time scan of the prepared solution of AuNPs at the room temperature shown in Fig. 1(b). The disappearance of the band at 410nm indicated that the precursor metal ions (chloroauric acid) are reduced to small metal ions in the presence of phenolic acids, flavonoids and other antioxidants present in *C.longa* extract, acting both as a reducing as well as stabilizing agents to form stable CL-AUNPs (MAHITHA *et al.,* 2013).

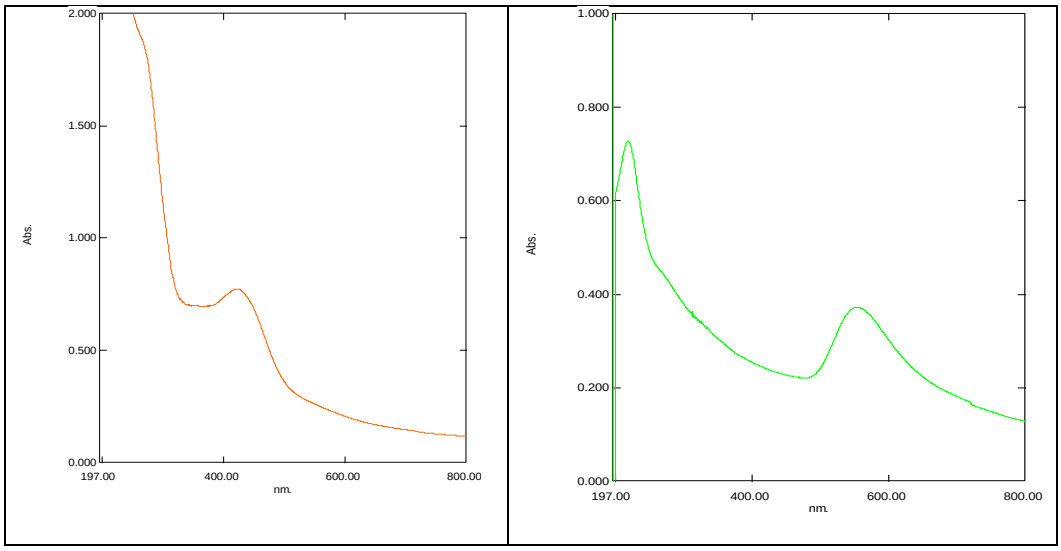

Fig. 1: UV-vis absorbance bands for *Curcuma longa* extract (left) and (b) Au-NPs forms (right) using *Curcuma longa* extract

Growth indices of *O.mossambicus*

The result of growth parameters of fishes are given in Table2.There is a significant increase in growth was observed in T3 group as compared to control and other experimental groups.

Table-2: The growth performance of *O. mossambicus*

Parameters	TD1	TD2	TD3
Initial weight (gm)	26.50±0.238	26.48±0.21	26.61±0.12
Final weight (gm)	29.95±0.12	30.55±0.12[a]	35.98±0.15[b]
Average wt. gain	3.45±±0.01	4.07±0.04[a]	9.37±0.01[b]
Weight gain (%)	13.01±0.15	15.37±0.19[a]	35.21±0.115[b]

Results are expressed as mean ± S.E. of triplicate groups of fish. The significant difference between groups was analyzed by One-Way ANOVA followed by Duncan's multiple range tests, a= significantly higher ($p<0.05$) than the controlTD1; b= significantly higher ($p<O.05$) than the control and TD2

Hemato-biochemical assays

The results related with haematological and biochemical parameters were obtained in the present investigation after the administration of different sources like plant extract of *C.longa*, plant extract with AuNPs and control are shown (Table-3). The Results Of the haematological analysis showed a significantly ($P<0.05$) different RBC level in the experimental groups T2 & T3 over the entire feeding period as compared to the control

(T1). The RBC count of T2 is significantly different from control on 60 days of experimental period. Significantly higher RBC count was recorded in group T3 where as lower RBC count was recorded in control.WBC count were significantly (P<0.05) different in experimental groups as compared to control. Significantly higher WBC count was observed in T2 among other experiment groups while lower WBC count was observed in T1. The haemoglobin content of T2 & T3 gropus varied significantly as compared to T1. Higher Hb level and hematocrit was observed in T3 as compared to other experiment groups. The serum biochemical analysis of the fish showed that the total serum protein, albumin, globulin level was highest in T3. It was significantly (P<0.05) high as compared to control T1 as well as other treatments. T2 treatment showed second best serum biochemical level which was also significantly (P<0.05) high as compared to T1. This result was supported by the study of founding that serum protein values were always higher in the fish treated with different immunostimulant than those in the control. Increase in the serum protein levels is thought to be associated with a stronger innate immune response in fish (WIEGERTJES *et al.,* 1996).

Table-3: Hemato-biochemical parameters of *O.mossambicus*

Parameters	**TD1**	**TD2**	**TD3**
Hb (g /dl)	8.53±0.08	10.73±0.17[a]	12.75±0.129[b]
RBC	3.15±0.34	4.8± 0.33[a]	5.13± 0.26[b]
WBC	3.25± 0.125	4.62. ±0.25[b]	4. 18± 0.17[b]
Hematocrit (%)	32.97± 3.31	36.16 ±4.04[a]	40.04 ± 3.74[b]
Total serum protein (g/dl)	1.96±0.19	2.13±0.12[a]	2.73±0.23[b]
Albumin(g/dl)	0.44± 0.12	0.57±0.02[a]	0.86±0.12[b]
Globulin content(gdl)	1.28± 0.10	1.59±0.07[a]	2.09 ±0.17[b]

Results are expressed as mean ± S.E. of triplicate groups of fish. The significant difference between groups was analyzed by One-Way ANOVA followed by Duncan's multiple range tests. a= significantly higher (p<0.05) than the controlTD1; b= significantly higher (p<O.05) than the control and TD2

In the present study dietary administration of biogenic gold nanoparticle enhanced the haematological indices of fish. Observations revealed that Tilapia fed with 4%green gold supplemented diet showed higher RBC, Hb and haematocritic value than plant extract treated group. Significant enhancement of haematological parameters may be due to enhanced erythropoietic centres in kidney /spleen, decreased erythroclasis and enhanced Fe metabolism. Enhanced RBC, WBC counts following biogenic gold fortified diet indicate the immunostimulant effect and anti infection properties of *C.longa*. Phytochemical analysis of *C.longa* revealed the presence of Flavnoids phytosterols, phenolic compounds, carbohydrates, and protein. This bioactive compound enhanced fish health by stimulating innate immunity (SHANKER *et al.,* 2004). A significant increase in total serum protein, globulin and albumin was observed in biogenic fortified diet as compared to other experimental group and control. Stronger non specific immune response is associated with marked increase in the total protein, albumin and globulin levels of fish.

CONCLUSION: In this study, a green approach for the synthesis of AuNPs using *C.longa* (turmeric) extract which is the simplest and efficient step to obtain AuNPs at room temperature without engaging any harmful chemicals as reducing and dispersing agent. The biomolecules present in the reductant, *C. longa*, were geared the reduction of gold to AuNPs. The overall result showed that biosynthesized AuNPs enhances growth, significant immunomodulatory effects and physiological changes with positive impact in

fresh water fish and this research provides helpful insights into the development of new immunomodulating nanomaterials for various aquaculture applications.

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REFERENCES

ABHIJITH, K.S, and THAKUR, M.S. 2012. Application of green synthesis of gold nanoparticle for sensitive detection of aflaxiin B1 based on metal enhanced fluroscence. *Analytical metods*, **4**(12): 4250-4256

CROMWELL, G. L. 1991. Antimicriobial agents. *In: E.R. Miller, D.E. Ullrey, A.J. Lewis (Ed.). Swine, nutrition, CRC Press, Boca Raton.* 297-315.

DHANYARAJ, D., F. SHINE, SHIBU JOSEPH, S. T., AND AKHILA THOMAS, A. 2017. Antibacterial Potential efficacy of *Curcuma longa* Mediated Biotransformed Gold Nanoparticles*. Proceedings of National seminar on Blue: Economy Fostering sustainable development and profitable resource utilization, August 2017*, pp33-35.

DUNCAN, D.B .1955. Multiple ranges and multiple F test. *Biometrics;* **11**: 1–42

HARDY, R. 1980.Fish feed formulation. *Lectures presented at the FAO/UNDP Training course in fish feed technology, held at the College of Fisheries. University of Washington, Seattle,* pp 233–240

HUBENTHAL, F. 2010. Nobel metal nanoparticles: synthesis and optical properties. *In: Andrews D L, Scholes G D, Wiederrecht G P, eds. Comprehensive Nanoscience and Technology. Boston: Elsevier;* 375-433.

JOHNSON, C. 2004. Influence of certain plant extract on growth and metabolism of teleosts (Anabas testudineus and Labeo rohita) and a mammal (*Rattus norvegicus*). *Ph.D thesis, University of Kerala, Thiruvananthapuram, Kerala, India***,** 46-47.

JOHNSON, C. W., TIMMSONS, D. L., and HALL, P. E.2002. Essential laboratory mathematics: concepts and applications for the chemical and clinical laboratory technician. Florence, USA: *Delmar Cengage Learning*.p. 268.

KHANNA, N. K. 1999. Turmeric –Nature's precious gift, *Curr. Sci*. **76**: 1358.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A.L. and RANDAL, R. J. 1951. Protein measurement with Folin- Phenol reagent*. J. Clin. Pathol*. 1**93**: 265-275.

MAHITHA, B. D., RAJU, T., MADHAVI, C. H., MAHALAKSHMI, N. J. and SUSHMA, 2013. Evaluation of antibacterial efficacy of phyto fabricated gold nanoparticles using *Bacopa monniera* plant extract. *Indian Journal of Advances in Chemical Science,* **1**(2): 94-98.

SHANKAR, S. S., RAI, A. and AHMAD, A. 2004. Rapid Synthesis of Au, Ag, And Bimetallic Au Core–Ag Shell Nanoparticles Using Neem (*Azadirachta Indica*) Leaf Broth. *Journal of Colloid And Interface Science*, **275**(2): 496–502.

SREELAKSHMI, C. H., NIDHI, GOEL, DATTA K. K., ANTHONY ADDLAGATTA and RAMESH UMMANNI, B. V. 2013. Green Synthesis of Curcumin Capped Gold Nanoparticles and Evaluation of Their Cytotoxicity. *Nanoscience and Nanotechnology Letters;* **5**: 1–8.

WIEGERTJES, G. F., STET, R. J. M. and VANMUISWINKEL, W. B. 1996. Immunogenetics of disease resistancein fish; a comparable approach. *Developmental and Comparative Immunology***. 20:** 365-381.

YU, D. G. 2007. Formation of colloidal silver nanoparticles stabilized by Na+poly (γ-glutamic acid)-silver nitrate complex via chemical reduction process. Colloids and Surfaces B: *Biointerfaces*; **59**(2): 171–178.

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ISOLATION OF THE EPIBIONT BACTERIA OF THE MARINE ALGAE *SARGASSUM WIGHTII* AND ITS CHARACTERIZATION

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ABSTRACT: Marine environment is a potential source for development of novel natural pharmaceuticals. Marine seaweeds and their associated bacteria produce bioactive compounds which have been found to be important for health promotion and disease prevention. The present study, focused on the isolation, characterization and screening of the antibiotic producing bacteria associated with *Sargassum wightii* from the coast of Kovalam,Thiruvananthapuram, Kerala. Ethyl acetate extracts of bacterial supernatant were screened for antibacterial activity. From the 8 isolates only one revealed antibacterial potential against the plant pathogen, *Alternaria alternata* which has been recorded causing leaf spot and other diseases on over 380 host species of plant. Identification of the active isolate up to genus level were carried out by morphological and biochemical analysis of the isolates. The most active isolates were identified up to species level by Phylogenetic analysis based on 16S rRNA gene sequencing. The Thin Layer Chromatographic assay was carried out for the most active isolate. Spot of the EPS developed on Thin Layer Chromatography was analyzed for its antimicrobial potential. Microbial communities associated with algae remain underexplored, despite their wide biodiversity and the fact that they differ markedly from those living freely in seawater. Further studies are required for the purification and chemical characterization of the bioactive products.

Key words: Antimicrobial potential, *Alternaria alternata*, epibiont, *Bacillus pumilus*, phylogenic tree, *Sargassum wightii*, secondary metabolites, supernatant

INTRODUCTION

Marine bacteria are a profound resource on the development of natural products and the medical sciences. The recent research reported that many bioactive natural products from marine invertebrates have striking similarities to metabolites of their associated microorganisms including bacteria. Bacteria and other micro-organisms are ubiquitous in the marine environment. They are taxonomically diverse, biologically active, and colonize all marine habitats, from the deep oceans to the shallowest estuaries. It has been estimated that the majority of bacteria in natural aquatic ecosystems are organized in biofilms (LEMOS *et al*., 1985).

The marine eukaryotes utilize their surface-associated bacteria to generate bioactive compounds in defense against antagonism and to look after the host against further colonization. These compounds may be derived from primary or rather secondary metabolism of these organisms (BÉRDY, 2005). The augmented use of chemical pesticides, many pathogenic strains have developed an improved level of resistance to existing pesticides. As a result, many pesticides that were not so long ago considered the first line of defense in the management of certain pathogens are no longer efficient (DAVIES, 1996) and consequently, there is an imperative demand for novel antimicrobials. Marine and terrestrial bacteria have many similarities, but the adaptations essential by organisms to live in a marine atmosphere, where chemical and physical environments fluctuate considerably from terrestrial environment, have resulted in synthesis of novel compounds to help in their survival. In fact, studies have shown that

marine bacteria are able to produce bioactive compounds that are not invented in terrestrial environment (FENICAL *et al*., 2006). Considering the above importance, the present study was undertaken to determine the antimicrobial potential of the secondary metabolites of epiphytic bacteria of the seaweed, *Sargassum wightii* collected from the Kovalam coast, Kerala against the fungal pathogen *Alternaria alternata*. *A alternata* causes black spot in many fruits and vegetables around the world. It is a latent fungus that develops during the cold storage of fruits, becoming visible during the marketing period thereby causing large postharvest losses.

MATERIALS AND METHODS

Seaweed samples were collected from the Kovalam coast (10°00'N 75°25'E), Thiruvananthapuram, Kerala. The samples were transported to the laboratory on ice. The surface of sample was washed with sterile sea water. The study was conducted on the period of 2015-2017 at Biotechnology and Microbiology Research Lab, Department of Zoology, Fatima Mata National College, Kollam, Kerala. A bacterial sample was taken from the surface with a sterile cotton swab. The serial diluted samples were spread on Zobell marine agar plates. The plates were incubated for 24-48 h at 37°C (ZHANG *et al*., 2007). The pure bacterial cultures obtained were maintained on Nutrient Agar slants. Overnight bacterial culture (100 ml) in marine broth was centrifuged at 7000 rpm for 20 min. The supernatant was collected and extracted with ethyl acetate. The organic layer was concentrated. The crude extract was used for bioassay against *A. alternata*. Antimicrobial activity was analyzed by agar well diffusion method. The culture of pathogen was collected from National Chemical Laboratory, Pune.

The isolated bacteria with antimicrobial activity were first identified to the genus level by observing their morphology and biochemical characteristics (MEARNS-SPRAGG *et al*., 1998). The bacteria with wide antimicrobial spectrum were identified up to species level by PCR amplification of the 16S rRNA gene, BLAST analysis, and comparison with sequences in the Gene Bank nucleotide database. The sequences were compared with known sequences in the Gene Bank nucleotide database. The crude extract of the most active epiphytic bacteria is made up to a concentration of 100 ml. The solution (2 µl) was submitted to TLC analysis on a silica gel plate. Spots of the Extra Polymeric Substance developed on the thin layer chromatography were used for antimicrobial assay using agar well diffusion method.

RESULTS AND DISCUSSION

The eight isolates obtained from *Sargassum wightii* from the Kovalam coast are represented as KS1, KS2, KS3, KS4, KS5, KS6, KS7 and KS8, respectively. The pure culture of the bacterial epibionts were maintained in agar slants and subsequently tested for their antimicrobial potential against the plant pathogen *A alternata* by agar well diffusion method. The inhibition zone obtained is shown in (Table-1) and (Photo plate-1).

Table -1: Zones of inhibition formed by epiphytic bacteria

Pathogen	**Isolated Epiphytic Bacteria(mm)**							
A alternata	KS 1	KS 2	KS 3	KS 4	KS 5	KS 6	KS 7	KS 8
	-	-	-	-	-	-	-	12

From the stains obtained from Kovalam only KS8 is antagonistic to *A alternata* (12mm). The isolates with antimicrobial potential were identified using morphology, Gram staining and Biochemical tests. The isolated strain is gram negative rods and positively reacted to

Methyl red, Voks-proskauer and catalase tests but negative to indole and oxidase tests. It utilizes citrate and ferment sucrose in the medium. But lactose fermentation is absent. The bacteria with wide antimicrobial potential was identified up to the species level by PCR amplification of the 16S rRNA gene, BLAST analysis, and comparison with sequences in the Gene Bank nucleotide database. Sequence analysis reveals that the strain KS8 is *Bacillus pumilus*. Phylogenic tree of *B pumilus* is given in (Fig- 1).

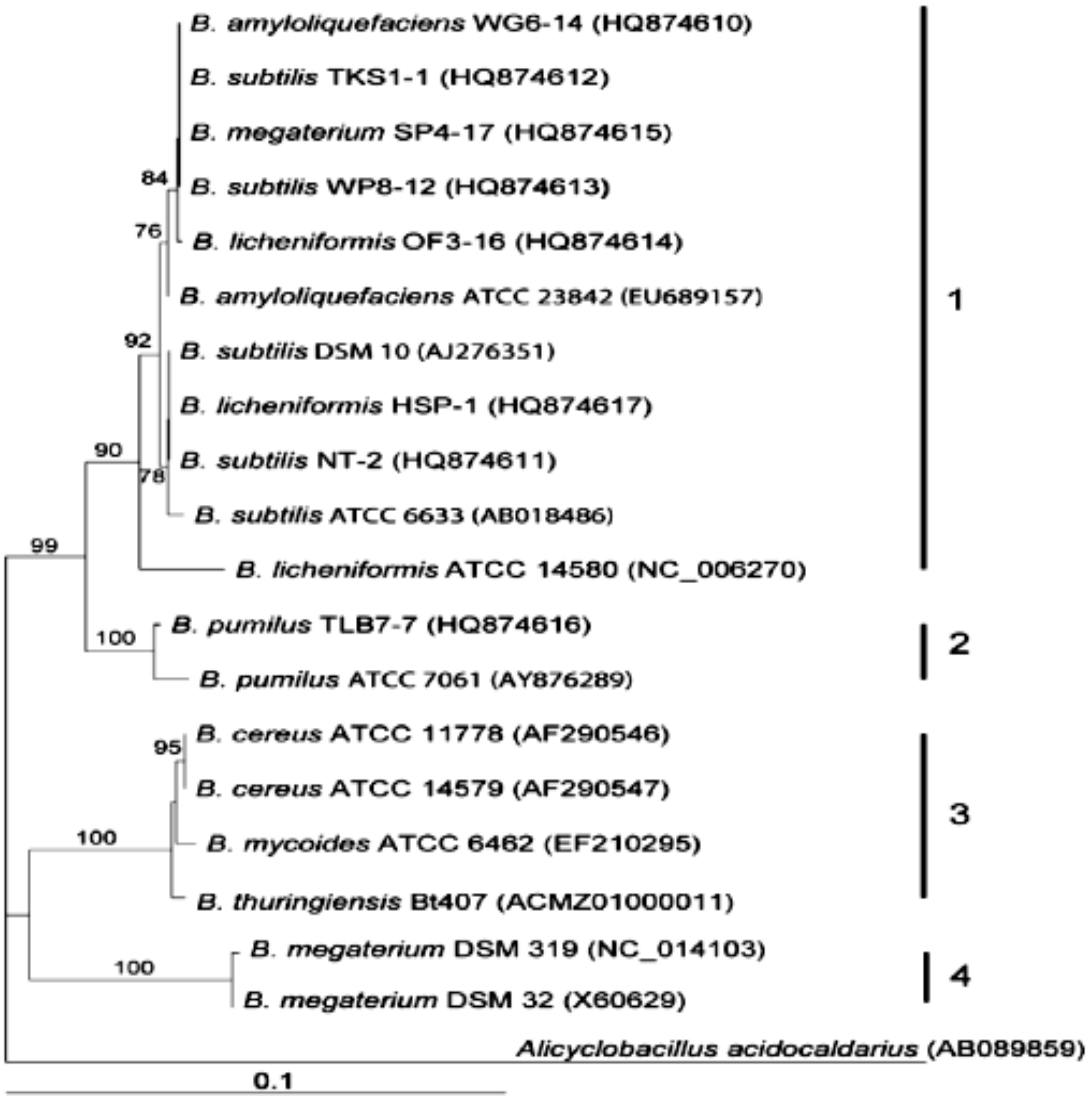

Fig.-1: Phylogenic Tree of *Bacillus pumilus* based on 16srRNA Sequences

Fig.-2: TLC of the Crude Extract of *B pumilus*

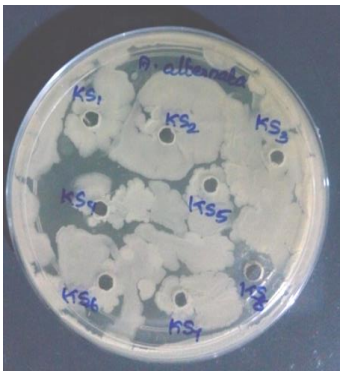

Photo plate- 1: Screening of antimicrobial against A alternate potential by agar well diffusion method

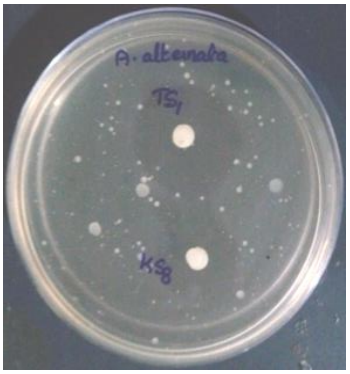

Photo plate-2: Screening of antimicrobial potential of EPS of *B pumilus* obtained by TLC

The crude extract of the *B pumilus* was submitted to TLC analysis. Spot obtained in TLC is shown in (Fig-2). Spots of the EPS developed on the thin layer chromatography were scraped from the plate assceptically and was used for antimicrobial assay using agar well diffusion method. The antimicrobial potential of EPS is shown in (Photo plate - 2).

Gram-negative bacilli from seaweed were obtained in the study. The bacterial diversity observed in the present study may be only a fraction of the total diversity of associated bacteria. The results indicate that certain species are selective in response to the pathogen. It can be stated that epiphytic bacteria of the marine algae used in this study had different defense mechanisms, which create an ecological and biotechnological interest in their antimicrobial activity. Development of economically feasible standard operating procedures for the production of extracts in large scale with reproducible antibacterial efficiency is necessary.

B. pumilus strain is used as an active ingredient in agricultural fungicides. Growth of the bacterium on plant roots prevents *Rhizoctonia* and *Fusarium* spores from germinating. Because of its ability to produce a large number of antimicrobial peptide, the genus *Bacillus* is becoming an interesting source to search for inhibitory substance (BONAR *et al*., 1998). The genus *Bacillus* includes aerobic endospore forming bacteria and is one of the largest sources of bioactive natural products (DOBRETSOV *et al*., 2006). Various research reports confirm that the *Bacillu*s species have a wide range of antimicrobial activities since they are used as antifungal, antibacterial, antiviral, antiameobocytic and antimycoplasma agents having a great potential for biopharmaceuticals and biotechnological applications (EGAN *et al*., 2008).

The information about the metabolic profile, antimicrobial activity and cytotoxicity of the selected *Bacillus* strains could be valuable in the search for new antibiotics and commercially useful therapeutic agents. Epiphytic bacteria from marine macro algae have been well studied in reference to their ecological importance with host organisms (CROFT *et al*., 2005). Apart from their association with seaweeds, *Bacillus* were previously isolated from sediments and seaweeds with antimicrobial properties (PRIETO *et al*., 2012). So far, more than 800 metabolites have been reported with various biological activities from the *Bacillus* genera. As observed in the present study the

potential isolate identified as *Bacillus* sp. showed remarkable activity against the tested pathogen, *A. alternata*.

REFERENCES

BONAR, D. B., WEINER, R.M. and COLWELL, R. R. 1986. Microbial–invertebrate Interactions and potential for biotechnology. *Microb Ecol*., **12**: 101–11.

CROFT, M. T., LAWRENCE, A. D., RAUX-DEERY, E., WARREN, M. J., AND SMITH, A. G. 2005. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature* **438**: 90–93.

DAVIES, J. 1996. Medicine – bacteria on the rampage. *Nature* **383**:219–220.

DOBRETSOV, S., DAHMS, H.U. and QIAN, P.Y. 2006. Inhibition of biofouling by marine microorganisms and their metabolites. *Biofouling*, **22**: 43–54.

EGAN, S., THOMAS, T., and KJELLEBERG, S. 2008. Unlocking the diversity and biotechnological potential of marine surface associated microbial communities. *Curr. Opin. Microbiol*., **11**: 219–225.

FENICAL, W., and JENSEN, P.R. (2006). Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.* **2**: 666–673.

MEARNS-SPRAGG, A., BREGU, M., BOYD, K.G. and BURGESS J.G.1998.Cross-species induction and enhancement of antimicrobial activity produced by epibiotic bacteria from marine algae and invertebrates, after exposure to terrestrial bacteria. *Lett. Appl. Microbiol*., **27**: 142-146.

PRIETO, M., SULLIVAN, L. O., MCLOUGHLIN, P., HUGHES, H., O'CONNOR, P. M., and GARDINER, G. E. (2012). Assessment of the bacteriocinogenic potential of marine bacteria reveals lichenicidin production by seaweed-derived *Bacillus* spp. *Mar.Drugs* , **10**: 2280–2299.

ZHANG, R., LIU, B.Z., LAU, S.C.K., KI, J.S. and QIAN, P.Y. 2007. Particle attached and free- living bacterial communities in a contrasting marine environment: Victoria Harbor, Hong Kong. *FEMS Microbiol Ecol* ., **61**: 496–495.

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IMPACTOF SUBLETHAL CONCENTRATIONS OF COPPER ON PROTEIN METABOLISM OF FISH, *OREOCHROMIS MOSSAMBICUS* (PETERS)

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ABSTRACT: Industries are the major sources of heavy metal pollution and it is released into water and soil. Heavy metals cause several ill effects to aquatic organisms and environment. In the present study, *Oreochromis mossambicus* were exposed to sublethal concentrations (1/16, 1/12, 1/8 and 1/4th of 96 h LC_{50} value) i.e. 3mg/L, 4mg/L, 6mg/L and 12mg/L of Copper Sulphate for four different exposure periods of 10, 20, 30 and 40 days. The proteins, Free amino acids, Aspartate amino transferase (AST) and Alanine amine transferase (ALT) levels in two different tissues such as muscle and liver were studied.Decreased tendency was observed in protein levels and an increased tendency was observed in Free amino acids, AST and ALT levels in all the vital tissues of fish exposed to Copper Sulphate over control. Proteins gradually decreased with increased exposure period of sublethal concentrations. Amino acids, AST and ALT levels gradually increased with increased exposure period and the increase was observed to be directly proportional to increased sublethal concentrations.

Key words: *Oreochromis mossambicus*, Copper sulphate, proteins, amino acids

INTRODUCTION

Water is an important natural resource which is essential for all living beings. The important water resources are ponds, lakes, rivers, streams, etc. The undesirable change in water that has harmful effect on the life of man and domestic plants and animals is called water pollution (RAVIKIRAN *et al*., 2015). The two most important factors that contribute to the deleterious effects of heavy metals as pollutants are their indestructible nature through bioremediation unlike organic pollutants and their tendency to accumulate in environment especially in the bottom sediments of aquatic habitats in association with organic and inorganic matter (SOBHA *et al.,* 2007). Copper occurs naturally within the environment. At low concentrations, it is an essential element both for plants and other organisms; however, large doses can be harmful. It is also used as an algaecide, an herbicide in irrigation and municipal water treatment system and for controlling phytoplankton in fish ponds and lakes as well as the herbicides used in aquatic weed control (CARBONELL and TARAZONA, 1993). QIU *et al*. (1997) showed that copper has toxic effects on the larval development of the barnacle, *Balanus amphitrite* and that molting was a more sensitive end-point than survival.

Studies on different biochemical parameters have proved useful in determining the adaptive and protective mechanisms of the body to resist the toxic effects of the toxic substances. Any alteration in biochemical parameters can result in serious outcomes in the form of various diseases in both the fish and its consumer. In the tissues proteins, carbohydrates and lipids play a major role as energy precursors for aquatic organisms exposed to stress conditions (RAMALINGAM, 1980). An alteration in biochemical and physiological changes in the crab, *Portunus pelagicus* due to Copper and Zinc has been reported by (HILMY*et al.*, 1988). KATTICARAN*et al*. (1995) also reported that the variation in carbohydrate and protein contents in the clam, *Sunetta*

scripta during its exposure to Copper. VILLALAN *et al.* (1988) observed that heavy metals altered protein, lipid and carbohydrate levels in the crab, *Thalamita crenata.* MAHARAJAN *et al*. (2012b & c) observed the biochemical changes of various tissues and haemolymph of spiny lobster, *Panulirus homarus* and the fresh water crab*, Paratelphus ajacquemontii* when exposed to sublethal doses of Copper. The development and growth of the fishes depend upon the DNA & RNA which serve as biochemical indices (BUCKLEY*et al*., 1980). Cellular enlargement and active protein synthesis are dependent on DNA & RNA content.

The protein content in the tissues of the animal plays a role in the metabolism.PALANIVELU *et al*. (2005) stated that the protein content of the cell may be considered as an important tool for evaluation of physiological standards. BEGAM and VIJAYARAGHAVAN (1996) observed that the protein depletion in the fish tissues indicates the physiological strategy in order to meet the energy demand and to adopt itself to the changed metabolic system which may lead to the stimulation of degradative processes like proteolysis and utilization of degraded products for increased energy metabolism. When any aquatic animal is exposed to polluted medium, a sudden stress is developed for which the animals should meet more energy demand to overcome the toxic stress MAHARAJAN *et al*. (2012a, 2014); PARURUCKUMANI *et al*. (2015a,b,c & d). VERMA *et al*. (1981) reported on the toxic effects of sublethal concentration of copper sulphate on certain biologically important enzymes in *Saccobranchus fossilis.*The purpose of present investigation is to determine the effect of copper sulphate on protein content, free amino acids, AST and ALT levels in certain tissues, i.e. muscle and liver of fish,*Oreochromis mossambicus.*

MATERIALS AND METHODS

The fish, *Oreochromis mossambicus* weighing bout 12- 14g used in the present study were procured from State fisheries culture tanks, Telangana. They were transported to the laboratory in oxygenated containers and treated with $KMnO_4$ to avoid dermal infection and acclimatized to laboratory conditions for 10 days. The fishes were fed with commercial feed once a day at a rate of 2% of body weight both before and during the experiment.Temperature was maintained at 27 ± 1°C and water in the containers was replaced by fresh water at every 24 hours. Biochemical parameters (Total proteins, Amino acids, AST and ALT) were estimated in two tissues, muscle and liver by exposing to four sub lethal concentrations of $CuSO_4$ i.e. 12 mg/L ($1/4^{th}$ of LC_{50}), 6 mg/L ($1/8^{th}$ of LC_{50}), 4 mg/L ($1/12^{th}$ of LC_{50}), and 3mg/L ($1/16^{th}$ of LC_{50}) for four different durations (10, 20, 30 and 40 days). Total proteins were estimated by Bradford method(1976). Amino acids were estimated by Moore and stein method (1954). AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase) levels were estimated by Bergmeyer method (1965). The above mentioned work was done in the Department of Zoology, Fish physiology lab, University College of Science, Osmania University, Hyderabad, Telangana for the period of 2014 to 2016.

RESULTS AND DISCUSSION

After exposing the test fish to different sublethal concentration of Copper sulphate ($CuSO_4$), 12 mg/L ($1/4^{th}$of LC_{50}), 6 mg/L ($1/8^{th}$ of LC_{50}), 4 mg/L ($1/12^{th}$of LC_{50}) and 3mg/L ($1/16^{th}$ of LC_{50}) for four different durations (10, 20, 30 and 40 days) of exposure on protein content, free amino acids, AST and ALT levels in muscle and liver of *O. mossambicus* fish were studied and the results were statically analyzed. The variations in levels of protein,Free amino acids, AST and ALT levels in different tissues given in tables (Table 1 to 8) in terms of mean with Standard error values over control. Proteins gradually decreased with increased exposure period of sublethal concentrations. Amino acids, AST and ALT levels gradually increased with increased

exposure period and the increase was observed to be directly proportional to increased sublethal concentrations.

The observation was made in both control and $CuSO_4$ exposed fish, muscle contains high levels of protein in comparison to liver tissue. At the end of the experiment the protein levels were decreased in both the tissues. The order of protein levels decrease in two tissues was observed as muscle >liver of fish. Decrease of protein levels was more at higher concentration of $CuSO_4$ (12mg/L) and higher duration (40days). In present study, free amino acids levels in different tissues like muscle andLiver were increased under sublethal exposure of $CuSO_4$. There was a significant increase in free amino acid levels in all tissues at all durations and in all sublethal doses were observed.The order of increase in different tissues when exposed to sublethal concentrations was observed as Liver > Muscle of fish.

The Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels in different tissues like muscle and liver were observed under sublethal exposure of $CuSO_4$.The mean values of AST with Standard error over the control were given in figure 5 and 6. There was a significant increase in AST levels in both the tissues at all durations and in all sublethal doses were observed.The order of increase in different tissues when exposed to sublethal concentrations was observed as liver >muscle of fish. There is a significant increase in ALT levels in both the tissues at all durations and in all sublethal doses were observed.The order of increase in different tissues was observed as liver >muscle of fish.

Table-1: Protein content in fish muscle after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		**Days of exposure**			
		10 days	**20 days**	**30 days**	**40 days**
Control	**Mean**	64.240	64.590	65.340	66.270
	SE	±1.200	±1.211	±1.144	±1.090
3mg/L	**Mean**	63.320^{NS}	62.170^{NS}	59.980^{NS}	57.940*
	SE	±1.239	±1.171	±1.311	±1.236
	%V	-1.43	-3.73	-8.20	-12.57
4mg/L	**Mean**	61.120^{NS}	58.720*	57.230**	54.260***
	SE	±1.386	±1.403	±1.176	±0.691
	%V	-4.85	-9.09	-12.41	-18.13
6mg/L	**Mean**	60.100^{NS}	55.280**	52.600***	50.370***
	SE	±1.260	±1.414	±0.890	±1.565
	%V	-6.45	-14.49	-19.49	-23.99
12mg/L	**Mean**	59.840^{NS}	53.340**	47.310***	42.320***
	SE	±1.772	±1.811	±1.544	±1.808
	%V	-6.86	-17.41	-27.46	-36.13

Each value is the Mean ±SE of six individual observations
Values are expressed as mg of protein / gram wet weight of tissueSE - Standard Error,
%V- Per cent variation.NS: Not Significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table-2: Protein content in fish Liver after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	50.300	50.490	51.480	52.250
	SE	±1.282	±1.274	±1.253	±1.189
3mg/L	Mean	50.370^{NS}	49.140^{NS}	48.220*	47.190**
	SE	±1.108	±1.187	±1.183	±1.141
	%V	0.12	-2.66	-6.34	-9.68
4mg/L	Mean	49.270^{NS}	48.260^{NS}	46.280*	44.220**
	SE	±1.067	±1.107	±1.186	±1.091
	%V	-2.06	-4.40	-10.10	-15.36
6mg/L	Mean	48.280^{NS}	45.290**	43.410**	41.280***
	SE	±1.123	±1.105	±1.201	±1.059
	%V	-4.03	-10.29	-15.68	-20.99
12mg/L	Mean	47.220*	43.280***	40.180***	37.260***
	SE	±1.124	±1.169	±1.141	±1.099
	%V	-6.14	-14.27	-21.94	-28.68

Each value is the Mean ±SE of six individual observations
Values are expressed as mg of protein / gram wet weight of tissueSE - Standard Error,
%V- Per cent Variation.NS : Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Table-3: Free Amino acid content in fish muscle after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	20.540	20.740	21.070	20.970
	SE	±0.343	±0.362	±0.557	±0.516
3mg/L	Mean	20.900^{NS}	21.120^{NS}	21.810^{NS}	22.820^{NS}
	SE	±0.338	±0.426	±0.629	±0.508
	%V	1.73	1.83	3.49	8.79
4mg/L	Mean	21.100^{NS}	21.720^{NS}	22.340^{NS}	22.920*
	SE	±0.466	±0.704	±0.510	±0.366
	%V	2.30	4.75	6.01	9.29
6mg/L	Mean	21.130^{NS}	22.010^{NS}	22.690*	23.340*
	SE	±0.566	±0.549	0.000	±0.285
	%V	2.84	6.12	7.67	11.30
12mg/L	Mean	21.380^{NS}	22.810**	25.080**	26.880***
	SE	±0.446	±0.066	±0.322	±0.301
	%V	4.05	10.01	19.03	28.14

Each value is the Mean ±SE of six individual observations
Values are expressed as mg of free amino acids/gram wet weight of tissue, SE-Standard Error
% V- Per cent variation. NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Table-4: Free Amino acid content in fish Liver after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	25.320	25.650	25.840	26.060
	SE	±0.165	±0.156	±0.112	±0.179
3mg/L	Mean	25.530^{NS}	25.840^{NS}	27.000**	28.280**
	SE	±0.178	±0.166	±0.217	±0.481
	%V	0.84	0.72	4.47	8.52
4mg/L	Mean	26.120^{NS}	27.210*	28.360**	30.420***
	SE	±0.314	±0.315	±0.222	±0.357
	%V	3.14	6.08	9.72	16.74
6mg/L	Mean	26.930*	28.190**	29.390**	32.130***
	SE	±0.374	±0.150	±0.412	±0.244
	%V	6.37	9.90	13.72	23.28
12mg/L	Mean	28.390**	30.530***	32.370***	35.260***
	SE	±0.329	±0.318	±0.414	±0.457
	%V	12.11	19.02	25.26	35.29

Each value is the Mean ±SE of six individual observations
Values are expressed as mg of free amino acids /gram wet weight of tissue SE-Standard Error
%V- Per cent variation. NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Table-5: AST level in fish Muscle after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	53.040	54.220	53.040	54.220
	SE	±1.021	±0.589	±1.021	±0.589
3mg/L	Mean	54.810^{NS}	56.580^{NS}	58.930**	60.120**
	SE	±1.021	±1.021	±0.5890	±1.021
	%V	3.33	4.34	11.11	10.87
4mg/L	Mean	56.580^{NS}	58.930**	60.110**	61.880**
	SE	±1.021	±0.589	±1.021	±1.021
	%V	6.66	8.69	13.33	14.13
6mg/L	Mean	57.760*	60.110**	63.060**	65.420***
	SE	±0.589	±1.021	±1.179	±1.021
	%V	8.89	10.87	15.88	20.65
12mg/L	Mean	61.290***	62.470***	64.830***	68.750***
	SE	±0.589	±0.589	±0.589	±0.853
	%V	15.55	15.21	18.18	26.80

Each value is the Mean ±SE of six individual observations
Values are expressed as µ moles of Pyruvate /mg / hourSE - Standard Error,
%V- Per cent variation. NS : Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Table-6: AST level in fish Liver after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	71.310	71.310	71.900	72.490
	SE	±0.589	±0.589	±0.589	±1.021
3mg/L	Mean	73.670^{NS}	76.030^{NS}	77.800*	80.740**
	SE	±0.589	±1.022	±1.021	±0.589
	%V	3.30	6.61	8.20	11.38
4mg/L	Mean	79.560*	81.330**	85.450***	91.350***
	SE	±1.019	±1.021	±0.589	±0.589
	%V	11.57	14.05	18.85	26.01
6mg/L	Mean	83.690**	86.630***	92.530***	100.190***
	SE	±0.589	±1.021	±0.589	±1.559
	%V	17.35	21.48	28.69	38.21
12mg/L	Mean	88.400***	96.070***	104.900***	117.280***
	SE	±1.021	±1.559	±1.559	±2.569
	%V	23.96	34.71	45.90	61.79

Each value is the Mean ±SE of six individual observations
Values are expressed as µ moles of Pyruvate /mg / hourSE - Standard Error,
%V- Per cent variation.NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Table-7: ALT level in fish muscle after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	37.128	33.592	33.592	33.128
	SE	±1.020	±0.590	±1.020	±0.680
3mg/L	Mean	35.950^{NS}	36.540^{NS}	38.310*	39.480**
	SE	±0.590	±0.590	±0.590	±0.590
	%V	-3.17	8.77	14.03	19.19
4mg/L	Mean	39.480*	40.070**	40.660*	43.020***
	SE	±0.590	±0.590	±1.020	±0.590
	%V	6.35	19.29	21.05	29.86
6mg/L	Mean	40.910*	43.690***	44.530***	46.560***
	SE	±1.320	±0.260	±0.750	±0.590
	%V	10.42	30.05	32.57	40.53
12mg/L	Mean	43.690***	44.530***	47.740***	49.500***
	SE	±0.260	±0.750	±1.020	±1.020
	%V	17.67	32.57	42.10	49.43

Each value is the Mean ±SE of six individual observations
Values are expressed as µ moles of Pyruvate /mg / hourSE - Standard Error,
%V- Per cent variation. NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Table-8: ALT level in fish liver after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	**20 days**	**30 days**	**40 days**
Control	**Mean**	53.630	55.400	54.810	54.810
	SE	±0.590	±0.590	±1.020	±1.020
3mg/L	**Mean**	55.400^{NS}	58.340^{NS}	60.700*	61.880*
	SE	±0.590	±1.020	±0.590	±1.020
	%V	3.29	5.32	10.75	12.90
4mg/L	**Mean**	60.110**	62.470***	64.830***	67.180***
	SE	±1.020	±0.590	±0.590	±1.020
	%V	12.08	12.76	18.28	22.58
6mg/L	**Mean**	64.230***	66.590***	68.950***	71.900***
	SE	±0.590	±0.590	±1.020	±0.590
	%V	19.77	20.21	25.80	31.18
12mg/L	**Mean**	69.540***	71.900***	75.430***	80.150***
	SE	±0.590	±0.590	±1.560	±0.590
	%V	29.67	29.78	37.63	46.23

Each value is the Mean ±SE of six individual observations
Values are expressed as µ moles of Pyruvate /mg / hourSE - Standard Error,
%V- Per cent variation.NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

At the end of the treatment of the fish, *O. mossambicus* to different sublethal concentrations of Copper sulphate ($CuSO_4$), proteins gradually found to decrease with increased exposure period of sublethal concentrations. Amino acids, AST and ALT levels increased with increased exposure period and the increase was observed to be directly proportional to increased sublethal concentrations. The observation was made in both control and $CuSO_4$ exposed fish, muscle contains high levels of protein in comparison to liver tissue. At the end of the experiment, the protein levels were decreased in both the tissues.The decrease in protein levels may be due to metabolic stress and proteolysis under toxic exposure of fish. Similar decrement in protein content was also observed when fish were exposed to Copper sulphate by MIKHAYLOVA *et al*. (1983).

The amino acids may be utilized for ATP production in two different ways. They could be converted to keto acid via transaminase and then fed to the TCA cycle. Alternatively they could be channeled into gluconeogenic pathway. Oxidation in Kreb's cycle meets the higher energy demands under Copper sulphate toxic impact (TOMARET *et al*., 2015). Amino acids are essential intermediate substances in the process of protein synthesis and its degradation products appear in the form of various nitrogenous compound (SOMAIAH *et al*., 2015). The present study revealed that, free amino acids levels in different tissues like muscle and liver were increased under sublethal exposure of $CuSO_4$.The increase in the free amino acid content is supported increased proteolytic action caused by the stress due to Copper sulphate. From the results, it is inferred that the increased free amino acid can be utilized for energy production (ATP) by feeding them into the TCA cycle through aminotransferase reaction.

The Aspartate aminotransferase (AST) levels in different tissues like muscle and liver were observed under sublethal exposure of $CuSO_4$.The Alanine

aminotransferase (ALT) levels in different tissues like muscle and liver were observed under sublethal exposure of $CuSO_4$. MCKIM *et al*. (1970) found that, sublethal concentration of Copper caused significant increase of pALT of *Salvelunus farttinalis* after 6 and 21 days of exposure. ADEL SHALABY (2000) reported that changes were produced in liver and muscle of common carp, *Cyprinus carpio* L. exposed to sublethal levels of Copper, Cadmium or Zinc alone or a combination of them for 7 to 30 days. The hepatic Aspartate aminotransferase (AST) in liver was increased. Also, hepatic Alanine aminotransferase (ALT) showed significant increase in fish.

Conclusion: The present investigation revealed that $CuSO_4$ caused alterations in biochemical parameters and enzymes of *Oerochromis mossambicus* might be caused by intoxication of heavy metal. It is concluded that the utilization of Copper sulphate should be minimized and should create awareness among the people about the toxicity of Copper sulphate on animals as well as on human. Since majority of heavy metals are released cumulatively and regularly, through the industrial and human activities, their residues are known to bioaccumulate in the tissues of fish and other animals, and transfer via food chain to the human bodies, they create risk to the health of the people who consume these fish seems to be considerable. The need to protect the people from undue exposure to the heavy metals through the food chain cannot be over emphasized.

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REFERENCES

BEGUM, G. and VIJAYARAGHAVAN, S. 1996. Alterations in protein metabolism of muscle tissue in the fish *Clarias batrachus* (Linn) by commercial grade dimethoate. *The Bulletin of Environmental Contamination and Toxicology* (BECT), **57**: 22.

BUCKLEY, L. J. 1980. Changes in ribonucleic acid, deoxy ribonucleic acid and protein content during ontogenesis in winter flounder (*Seudopleuronectes americanus*) and the effect of starvation. *Fisher. Bull., U.S*., **77**: 703-708.

CARBONELL, G. and TARAZONA, J. V. 1993. A proposed method to diagnose acute copper poisoning in cultured rainbow trout (*Oncorhynchus mykiss*). *The Science of the total Environment*, **2**: 1329-1334.

FINNEY, D. J. 1971. Probit Analysis, (3rd edn). *Cambridge University Press, Cambridge, pp.269-282.*

HILMY, A. M., N. F. A. EL-HAMID and GHAZALY, K. S. 198). Biochemical and physiological changes in the tissues and serum of both sexes in *Portunus pelagicus* (L) following acute exposures to zinc and copper. *Folia Morphologica*, (Prague) **36**: 79-94.

HOLLAND, G. A., LASATER,J. E., NEUMANN, E. D. and ELDRIDGE, W. E. 1960-1964. Toxic effects of Organic and Inorganic Pollutants on Young Salmon and Trout. *State of Washington, Department of Fisheries, Research Bulletin* No. 5.pp.278

RAVIKIRAN, K. and KULKARNI, R. S. 2015. Nucleic Acid Content in Male Fresh Water Fish *N. notopterus* Exposed to Copper Sulphate. *International Letters of Natural Sciences* **33**: 1-8,

KARRASOMAIAH, P. V. V., SATISH, K., SUNITA, N. and BARATHAJYOTHI, 2015. Studies on the Biochemical Responses in the Tissues of Freshwater Fish *Labeorohita*Exposed to the Organophosphate, Phenthoate, *Int. J. Pharm. Sci. Rev. Res*., **32**(2): 108-115

KATTICARAN, C. M., MOHAMMED SALIB. K. Y. and JOSEPH, P. S. 1995. Copper induced alterations in total carbohydrate and protein levels in bivalve, *Sunetta scripta* (Bivalvia). *Indian Journal of Marine Science* **24**: 171-174.

MAHARAJAN, A., RAJALAKSHMI, S., VIJAYAKUMARAN, M. and KUMARASAMY, P. 2012. Sublethaleffect of copper toxicity against histopathological changes in the spiny lobster, *Panulirus homarus* (Linnaeus, 1758). *Biological Trace Element Research 145: 201-210. doi:10.4038/sljas.v17i0.6853.*

MAHARAJAN, A., NEELAKANDAMOORTHY, N. and KUMARASAMY. P. 2012. Impact of Profenofoson oxygen consumption and gill histopathology of the Fresh water crab, *Paratelphus ajacquemontii* (Rathbun) *Res. Rev.: J. Toxicol.,* **2**(2): 46-55.

MCKIM, J. M., CHRISTENSEN, G. M. and HUNT, E. P. 1970.Changes in the blood of brook trout (*Salvelinus fontinalis*) after short-term and long-term exposure to copper.*Journal of the Fisheries Board of Canada,* **27**(10), 1883-1889.

MIKHAYLOVA, L.V. 1983. Complex formation between the water- soluble petroleum fractionand blood and liver proteins of carp *Cyprinus carpio. Journal of Ichthyology*, **23**(3): 154- 158.

MURTY, A. S. and DEVI, A. P. 1982. The effect of endosulfan and its isomers on tissue protein, glycogen and lipid in the fish, *Channa punctatus*. *Pesticide Biochemistry and Physiology*, **17**: 280-286.

PALANIVELU, V., VIJAYAVEL, K., BALASUBRAMANIAN, S. and BALASUBRAMANIAN, M. P. 2005. Influence of insecticidal derivative (Cartap hydrochloride) from the marine polycheate on certain enzyme systems of the fresh water fish *Oreochromis mossambicus*. *Journal of Environmental Biology*, **26**: 191-195.

PARURUCKUMANI, P. S., MAHARAJAN, A., GANAPIRIYA. V., NARAYANASWAMY. Y. AND RAJA JEYASEKAR, R. (2015). Surface ultrastructural changes in the gill and liver tissue of Asian sea bass, *Latescalcarifer* (Bloch) exposed to copper, *Biological Trace Element Research,* **168**: 500-507.

PARURUCKUMANI, P. S., MAHARAJAN, A., GANAPIRIYA, V. AND KUMARASAMY, P. 2015. Bioaccumulation and ultrastructural alterations of gill and liver in Asian sea bass, *Lates calcarifer* (Bloch) in sub lethal copper exposure, *Aquatic Living Resources*. **28:** 33-44.

QIU, J. W., GOSSELIN, L. and QIAN, P. Y. 1997. Effects of short-term food availability on larval development in the barnacle *Balanus amphitrite*. *Marine Ecology Progress Series* **161**: 83–89.

RAMALINGAM, K. 1980. Studies on the effects of sublethal concentration of a few toxicants on biochemistry, physiology and histology of *Tilapia mossambica* (Peters), *Ph.D. Thesis, University of Madras, Chennai.*pp. 1-141

RAVIKIRAN, K. and KULAKARNI, R. S. 2015. Nuclic Acid content in male fresh water fish *N.Notopterus* exposed to Copper Sulphate, *International Letters of Natural science*, **6**:1-8.

SHALABY, A. M. E. 2000. Sublethal of heavy metals copper, cadmium and zinc alone or in combinations on enzymes activities of common carp *(Cyprinus carpio L.). Egypt J. AquatBiol Fish* **4**(2): 229–246

SOBHA, K., POORNIMA, A, HARINI, P. and VEERAIAH, K. 2007. A study on biochemical changes in the fresh water fish, *Catla catla* (Hamilton) exposed to the heavy metal toxicant cadmium chloride. *Kathmandu University Journal of Science, Engineering and Technology Vol., I, No. IV, August, 2007*.

TOMAR, A., VYAS, R., BAINERJEE, S. and ABHISHEK, G. 2015. Effect of Copper Sulphate on the Regulation of Nitrogen Metabolism in the fish, *Rita rita, J. Fisheries Livest. Prod.* **3**: 146.

VERMA, S. P., YEATS, R. S., HAQ, B. U., BARRON, J. A., BUKRY, D., CROUCH, J., DENHAM, C., DOUGLAS, A. G., GRECHIN, V. I., LEINEN, M., NIEM, A. R.,

PISCIOTTO, K. A., POORE, R. Z., SHIBATA, T. and WOLFART, R. 1981. K, Rb, Cs, Ba, and Sr contents &Sr isotope ratios of igneous rocks from Deep Sea Drilling Project Leg 63. *Initial Reports of the Deep Sea Drilling Project* **63**: 733-738.

VILLALAN, P., NARAYANAN, K. R., AJMAL KHAN, S. and NATARAJAN, R. 1988. Proximate composition of muscle, hepatopancreas and gill in the copper exposed estuarine crab *Thalamita crenata* (Latreille). *Proc. II National Symposium of Ecotoxicology,* 55–59.

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