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PLANT VOLATILES AND INSECT HERBIVORE INTERACTIONS: AN OVERVIEW

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ABSTRACT: Plants respond to insect herbivores in different ways, of which production of volatile organic compounds (VOCs) is one phenomenon. Herbivorous insects respond to changes in plant odour production, which influence the herbivore fitness costs of plant volatile production on insect growth and survival. Plant odours are comprised of mixtures of volatiles produced by different biosynthetic pathways. These odours provide species-specific signals to herbivorous insects, which are attracted to their host plants to feed or lay eggs. The number and relative concentrations of volatile compounds released by various plant organs can vary according to factors such as plant age, flowering status, nutritional status and damage through insect herbivory, thereby carrying information relating to plant quality. The defensive reactions of the plant, following physical injury by the herbivore, are influenced by a multitude of factors including, but not necessarily limited to, the elicitors and numerous other herbivore-associated molecules, as well as microbes on the plant surface that may alter plant defensive pathways. Differences in plant volatile expression in *C. formicarius* resistant and susceptible genotypes of sweet potato were present. A compound, 2-(2-butylcyclopropyl)-cyclopropanenonanoic acid methyl ester eluted from flower headspace volatiles of 'Howrah' genotype emitted as 9,12-(*Z,Z*)-octadecadienoic acid from the storage root periderm. 9,12-(*Z,Z*)-octadecadienoic acid is a precursor for production of several short-chain aromatic compounds through lipoxygenase (LOX) pathway, and is believed to operate in the storage roots. *Myzus persicae* and *B. tabaci* have similar roles in vectoring sweet potato viruses as both insects suppress the same types of volatile compounds. Many plants respond to herbivory by activating induced resistance mechanisms. Induced resistance mechanisms include the production of both volatile and nonvolatile secondary metabolites. These herbivore-induced compounds can be a key factor in mediating herbivore–herbivore interactions and in structuring insect communities. Insects also suppress plant volatiles by activating their own defense mechanisms.

Key words: Plant-insect interactions, chemical ecology, herbivory, natural enemies, volatiles, induced defenses, elicitors, suppressors, priming

INTRODUCTION

Plants produce distinct blends of herbivore-induced volatiles, and these are known to attract a range of herbivore enemies, including predators and parasitoids drawn from five insect orders, plus predatory mites, nematodes and birds. Much of the research on volatile-mediated attraction of herbivore enemies to damaged plants has centered on the ecological and evolutionary significance of volatile emissions to plants. Herbivorous insects respond to changes in plant odour production, which influence the herbivore fitness costs of plant volatile production on insect growth and survival. In choice tests, fewer eggs were laid on transgenic plants *Nicotiana tabacum* compared with non-transformed controls, indicating that increased (S)-linalool emissions have a deterrent effect on *Helicoverpa armigera* oviposition. Larval survival and larval mass after feeding on transgenic leaves, however, was comparable to non-transformed controls. (S)-linalool, whether in volatile or sequestered form, does not appear to have a direct effect on offspring fitness in this moth (MCCALLUM *et al*., 2011). These odours provide species-specific signals to herbivorous insects, which are attracted to their host plants to feed or lay eggs. The number and relative concentrations of volatile compounds released by

various plant organs vary according to factors such as plant age, flowering status, nutritional status and damage through insect herbivory, thereby carrying information relating to plant quality.

Leaves, flowers and storage roots of sweet potato emit different types of volatile compounds which have influenced the behavior of sweet potato weevil *Cylas formicarius* (KORADA *et al.*, 2010). The flower and root volatiles are more attractive to female *C. formicarius*. Synthetic volatile blends of flowering rice panicles composed of geranyl acetone, β-caryophyllene, *n*-decanal, methyl salicylate, β-elemene and *n*-tridecene attracted females *Stenotus rubrovittatus* (Heteroptera: Miridae). The synthetic blend of volatiles was just as attractive as natural flowering rice panicles to females. β-caryophyllene and β-elemene are common active compounds responsible for attractiveness of flowering rice panicles and *Scirpus juncoides* spikelets although some of the other volatile components act synergistically with these two compounds in natural plant odours (HORI and NAMATAME, 2013). Rice stink bug, *Oebalus pugnax* F. (RSB) feeding induced volatiles production in different RSB host grasses and rice varieties, and may help explain RSB movement to heading rice. Limonene and methyl salicylate (MeSA) were found in varying amounts from panicles of host grasses and rice. RSB feeding induced caryophyllene production from panicles of only rice and vaseygrass. Limonene was produced in higher amounts in the RSB-resistant rice cultivar 'Kaybonnet' than in more RSB-susceptible 'Cocodrie' and 'Bengal' (SINGH *et al.,* 2006).

Linalool is a common floral volatile in many moth pollinated plants and has been shown to have both attractant (RAGUSO *et al*., 2003; SUCKLING *et al*., 1996) and deterrent (KESSLER and BALDWIN, 2001) properties in ovipositing Lepidoptera. Linalool is a major component of the floral odour of tobacco, a preferred host of *H. armigera* and is also produced in small quantities from vegetative tissue volatile blends in response to herbivore attack. At high concentrations, linalool can be toxic to a variety of insect species (ABDELGALEILV *et al*., 2009; CHANG *et al*., 2009; PHILLIPS *et al*., 2010), affecting mortality, growth, activity and feeding in lepidopteran larvae (RAJWINDER *et al*., 2010; SINGH *et al*., 2009). This volatile occurs naturally in two isomeric forms, and a recent study using artificial odour blends has suggested that the *R* and *S* isomers of linalool may be perceived differently by the moth *Manduca sexta*, with both isomers being attractive to nectar feeding moths, but only the *R* isomer deterring ovipositing moths (REISENMAN *et al*., 2010).

PLANT VOLATILE COMPOUNDS

Plant volatiles are comprised of mixtures of volatiles produced by different biosynthetic pathways. Plants produce a large range of metabolites that are volatile because of their high vapor pressure under standard conditions. Aside from simple gases, such as O_2, CO_2, water vapor and ethylene, over 1700 volatiles are reported from plants (KNUDSEN *et al.,* 2006), but only a fraction of these are emitted by individual plants after herbivore damage. These can be grouped into four categories.

Terpenes

Comprising the largest class of plant volatiles, terpenes or terpenoids are classified by the number of branched C5 units in their structures. Major terpene volatiles emitted from vegetative tissue include the C5 compound isoprene (one C5 unit), C10 monoterpenes, such as (E)-β-ocimene and linalool (two C5 units), and C15 sesquiterpenes, such as (E)-β-caryophyllene and (E,E)-α-farnesene (three C5 units). Two terpenes that occur frequently after herbivore damage have irregular structures, the C11 (E)-4,8-dimethyl-

1,3,7-nonatriene (DMNT) and the C16 (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT).

Fatty acid derivatives

The oxidation of fatty acids leads to the formation of a large family of volatile derivatives. After herbivore damage, sequential lipoxygenase and hydroperoxidelyase action results in the production of C6 compounds, such as (E)-2-hexenal, (Z)-3-hexenal, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate, called GLVs because they impart the typical odor of green leaves.

Aromatic compounds

The metabolism of phenylalanine leads to a group of compounds with simple aromatic rings and C1–C3 side chains, whereas an offshoot of tryptophan biosynthesis leads to indole derivatives. The most important representatives of this group after herbivore damage are methyl salicylate and indole.

Amino acid derivatives

After herbivore damage, various amines, oximes, nitriles, isothiocyanates and sulfides are released that are produced from amino acids. These compounds are often not as well recovered in standard headspace collections as terpenes, GLVs and aromatics, and may be more abundant than is currently realized.

HERBIVORE PRODUCED ELICITORS

When an insect begins to damage a leaf, many biochemical reactions that precedes changes in plant defense gene expression. The defense signaling that occurs following this physical injury is ultimately shaped by the interactions of a multitude of potential players-the plant, the insect and their associated microbes.FAC elicitors are clearly not the only compounds produced by chewing caterpillars with the potential to induce defensive reactions by plants or otherwise affect their biochemistry. SCHMELZ *et al.* (2006) demonstrated induced volatile release in cowpea seedlings fed on by fall armyworm caterpillars. While volicitin and N-linolenoyl-L-glutamine were found in the regurgitant of these caterpillars, they failed to stimulate any significant response. This finding led to the discovery of inceptins, a new class of peptide elicitors of cowpea volatiles from fall armyworm regurgitant (SCHMELZ *et al*., 2006). Inceptin is a disulfide bridged peptide (+ICDINGVCVDA_) derived from the chloroplastic ATP synthase of the plant on which the caterpillars feed. Amount as low as 1 fmol per leaf induces increased levels of jasmonic acid and salicylic acid in cowpea leaves and release of ethylene and terpenoid volatiles (SCHMELZ *et al.,* 2006).

Another class of insect herbivore-produced, fatty acid elicitors of plant volatiles was first isolated and identified from the oral secretions of the grasshopper species *Schistocerca americana*, (ALBORN *et al.,* 2007). They are apparently common in the Orthoptera suborder Caelifera, and thus have been named caeliferins. The caeliferin fatty acid chain varies in length from 15 to 19 carbons and may be saturated or monounsaturated. Caeliferin A has sulfated hydroxyls on the a- and v-carbons of the fatty acid chain, while the caeliferin B molecules are sulfated a-hydroxyl diacids with the v-carboxyl conjugated to glycine, similar to the FACs. In *S. americana* the 16-carbon compounds are most abundant in each class. Caeliferin A16:1 is most active in inducing volatile organic compound (VOC) emission, while caeliferin A16:0 and caeliferin B16:0 are considerably less active. Other compounds in this family, including caeliferin A17:0 and 18:0, also appeared to have a low level of activity on corn seedlings.

The only known insect-produced elicitors of direct plant defenses are the bruchins, mono- and bis-(3-hydroxypropanoate) esters of C22 and C24 α,ω-diols. These compounds, deposited on pea, *Pisum sativum* L., pods during oviposition by both pea weevils, *Bruchus pisorum* L., and cowpea weevils, *Callosobruchus maculatus* l., induce the plant to form callus tissue under the egg and thus deter the hatched larvae from burrowing directly into the pea pod. Bruchins are active in amounts as low as 0.5 pg (1 fmol). There is tremendous variability in responses of plants to feeding damage by insect herbivores.

SPECIFICITY IN PLANT VOLATILE EMISSION

Each plant species emit a distinct blend of volatile compounds, and thus be recognizable to herbivores and their enemies. However, perusal of the major herbivore-induced volatiles shows that the same constituents are released by most plant species, irrespective of their taxonomic affinities. For example, the monoterpenes (E)-β-ocimene and linalool, the sesquiterpenes (E, E)-α-farnesene and (E)-β-caryophyllene, the C11 homoterpene (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and the fatty acid derivatives known as green leaf volatiles (GLVs), including (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate, are frequent components of volatile blends released after herbivore damage from a wide range of plant species. KORADA *et al*. (2010) SAMANTARAY and KORADA (2016) found that sweet potato weevil differently responds to volatiles emitted from different varieties of sweet potato and different plant parts (leaves, flowers, storage roots). Nevertheless, the relative amounts of these substances vary greatly among species and there are typically many differences in less abundant compounds that could contribute to specificity. If these differences are perceived by herbivore enemies, they could facilitate species recognition. Within a single species, plant volatile emission can vary with the herbivore present, as noted many years ago by Dicke and colleagues (DICKE *et al*., 1993). This variation provides herbivore enemies with valuable information on the identity of prey or hosts available on a plant and their feeding guilds. For example, *Brassica rapa* (turnip rape) plants damaged by the root herbivore *Delia radicum* (cabbage rootfly) emits a distinct blend of volatiles from their aboveground tissue that differs significantly from the blend that is released when the plants are attacked above ground by caterpillars of *Pieris brassicae* (large cabbage white butterfly). 4-Methyltridecane and salicylaldehyde are dominant compounds in the blend of *D. radicum*-damaged plants, whereas methyl salicylate is characteristic for *P. brassicae*-damaged cabbage. When roots and shoots are attacked simultaneously, the GLV hexyl acetate is released in high relative amounts.

Despite these impressive examples of specificity in the herbivore-induced emission of plant volatiles, there are several reports in which different herbivore species, feeding guilds, developmental stages and number of attackers were not found to alter volatile emission significantly (HARE, 2011; HARE and SUN, 2011; KESSLER and BALDWIN, 2011). For example, the spectrum of *Nicotiana attenuate* (coyote tobacco) volatiles induced by herbivory from a lepidopteran, *Manduca quinquemaculata* (five-spotted hawkmoth), a beetle, *Epitrix hirtipennis* (tobacco flea beetle) and an hemipteran, *Tupiocoris notatus* (suckfly) is similar with compounds being released in only slightly different proportions (KESSLER and BALDWIN, 2011).

Volatilome of scented rice cultivars is more complex than non-scented rice cultivar. N-heterocyclic class was the major distinguishing class between scented from non-scented rice. A total of 14 compounds including, 2AP were detected specifically in scented rice cultivars. Maximum number of compounds were synthesized at seedling stage and decreased gradually at reproductive and maturity. The seedling stage is an

active phase of development where maximum number green leaf volatiles were synthesized which are known to act as defense molecules for protection of young plant parts. Among the 14 odor active compounds (OACs), 10 OACs were accumulated at higher concentrations significantly in scented rice cultivars and contribute in the aroma. 2AP content was highest in mature grains followed by at booting stage. Gene expression analysis revealed that reduced expression of *betaine aldehyde dehydrogenase* 2 (*badh*2) and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) and elevated level of *triose phosphate isomerase* (*TPI*) and Δ*1-Pyrolline-5-carboxylic acid synthetase*(*P5CS*) transcript enhances 2AP accumulation. Most diverse compounds were synthesized at seedling stage and OACs were accumulated more at flowering followed by seedling stage. Distinct accumulation pattern exists for 2AP and other aroma volatiles at various developmental stages (HINGE *et al.,* 2016).

HERIBIVORE INDUCED VOLATILES ABOVE GROUND

Grasses consistently produce terpenes through lipoxygenase pathway (DEGENHARDT, 2009) in response to herbivore damage. The amount and composition of the volatile signal emitted by the plant is dependent on the type of herbivore cue. In *Nicotiana attenuata*, an *Ile* conjugate of jasmonic acid is crucial for the induction of nicotine production after herbivore damage to the plant. This conjugate interacts with the factor COI, an F-box protein essential for plant signaling processes (PASCHOLD *et al*., 2007). In Arabidopsis (*Arabidopsis thaliana*), a complex of jasmonic acid conjugate and COI protein targets a repressor of the JAZ family for degradation by the 26S proteasome and thereby activates genes of plant defense (THINES *et al*., 2007). Further transcription factors involved may be those of the WRKYfamily, which have already been implicated in the jasmonate-dependent expression of a terpenesynthase in cotton (*Gossypium hirsutum*) responsible for the production volatile (+)-δ-cadinene (XU *et al.,* 2004).In rice, three herbivore-induced terpenesynthases are sufficient to produce the majority of the terpene volatiles. The terpene blends of maize are formed by at least six sesquiterpenesynthases. Three of these enzymes, TPS1, TPS10, andTPS23, are strongly induced by herbivore damage and produce the major sesquiterpene components of herbivore-induced volatiles. The seterpenesynthases can also be induced by jasmonic acid treatment of the plant.

The composition and biological activity of volatiles also changes over time after herbivore attack. At the onset of herbivory, the blend is dominated by the green leaf volatiles, (E)-2-hexenal and (Z)-3-hexen-1-ol, while mono- and sesquiterpenes appear about 2 h later. In grasses, the species of attacking herbivore does not seem to affect the composition of the volatiles very much. Only slight differences between the volatile blends were reported in maize attacked by the lepidopteran larvae *S. littoralis* and *Ostrinia nubilalis* (TURLINGS *et al*., 1998), and rice infested with *Pseudaletia separata* and *Helicoverpa armigera* (YAN and WANG, 2006) possess no distinct differences in their volatile profiles.

For some herbivore enemies, however, minor differences between the volatile blends that are undetectable by gas chromatography can strongly affect host-seeking behavior. Electrophysiological studies indicated that the female SPWs showed a high depolarization of 0.2 mV to a compound emitted from Howrah flower extracts at 11.9 min, whereas the male antenna did not respond to the same extract with the same GC–EAD programme, thus providing evidence that female SPWs could detect specific compounds, even when they are present/emitted in very minute quantities (KORADA *et al.,* 2013a).

HERBIVORE INDUCED VOLATILES BELOW GROUND

Despite being covered by soil, roots are also subject to attack by herbivores. Although very little is known about indirect defense mechanisms below ground, it was often assumed that entomopathogenic nematodes are attracted to damaged roots via chemical cues. Recently, such a below-ground defense against arthropods was elucidated in detail. The defense is targeted against larvae of the beetle, *Diabrotica virgifera virgifera* (western corn rootworm), an important root pest of maize. In response to feeding by *D. v. virgifera* larvae, maize roots release a signal that strongly attracts the entomopathogenic nematode *Heterorhabditis megidis* (RASMANN *et al*., 2005). The attractive signal was identified as (E)-β-caryophyllene. Most North American maize lines do not release (E)-β-caryophyllene from their roots, whereas many European lines and the closest wild relatives of maize, teosinte, do so in response to *D. v. virgifera* attack. Field experiments showed a 5-fold higher nematode infection rate of *D. v.virgifera* larvae on a maize variety that produces the signal than on a variety that does not (RASMANN *et al*., 2005). The (E)-β-caryophyllene signal is produced by the (E)-β-caryophyllene synthase TPS23, which is independently regulated in leaves and roots in response to damage by different herbivores. Above and below ground, the signal is involved in the defense against herbivores with completely different sites and modes of attack. The ability of TPS23 to produce (E)-β-caryophyllene is widely distributed among the wild relatives of maize and was shown to be under positive selection pressure. However, the loss of (E)-β-caryophyllene production in most North American maize varieties is not due to inactive alleles of the TPS23 gene itself, but caused by an alteration of the signal transduction network that abolishes herbivore induced gene transcription (KOLLNER *et al*., 2008). (E)-β-caryophyllene diffuses more efficiently through soil than most other sesquiterpenes, making it a rather specific defense signal against soil herbivores (HILTPOLD and TURLINGS, 2008).

Within the same plant or crop, differences in chemical compounds they emit in space, differ between genotypes or varieties. Differences in plant volatile expression in *C. formicarius* resistant and susceptible genotypes of sweet potato were present. A compound, 2-(2-butylcyclopropyl)-cyclopropanenonanoic acid methyl ester eluted from flower headspace volatiles of ‘Howrah’ genotype emitted as 9,12-(*Z,Z*)-octadecadienoic acid from the storage root periderm. 9,12-(*Z,Z*)-octadecadienoic acid is a precursor for production of several short-chain aromatic compounds through lipoxygenase (LOX) pathway, and is believed to operate in the storage roots (KORADA *et al.,* 2013a). These compounds playing a role in *C. formicarius* resistance in ‘Howrah’ and ‘BX 86’ genotypes, were absent in SPW susceptible-genotype ‘Kishan’. KORADA *et al.* (2013a) proposed that the esters, i.e. cyclopropane fatty acid esters as a ‘diagnostic chemical marker’ to identify sweet potato weevil resistance in genotypes of sweet potato.

Plants and storage roots of three sweet potato varieties, namely, Beaugard, Evangeline and Murasakhi emitted different types of compounds when infested by sweet potato weevil (KORADA, 2012). Uninfested sweet potato plants emitted more number of volatile compounds than when the plants were infested by female sweet potato weevil, *C. formicarius*. Female weevil is able to supress emission of trans-2-hexenyl acetate, β-ocimene, para-cymene, 1-methyl naphthalene, 2-hydroxy, phenyl benzoic acid and α-humulene from sweet potato plants. The female weevil also produced two compounds cyperene and geranyl acetate (KORADA, 2012). A difference between volatile compounds from sweet potato storage roots infested by male and female *C. formicarius* was observed (KORADA, 2012). The number of volatile compounds emitted from female infested storage roots was more than the male infested roots. In case of Beaugard, female produced more no. of compounds than either healthy tubers or male induced

tubers. Female weevils exclusively produced sabinene and (*E*)-2-decenal from roots, whereas they are not found in either healthy tubers or male infested tubers. In case of Evangeline, both male and female infested tubers suppressed some of the compounds those emitted from healthy tubers. There are not much differences in compounds released from either healthy or infested storage roots in case of var. Murasakhi. Murasakhi roots produced entirely a new compound gernanyl acetate and (*E*)-2-decenal, both are not produced by uninfested tubers (KORADA, 2012).

PLANT VOLATILES IN RELATION TO HERBIVORE ENEMY COMPLEX

The blends of volatiles released from damaged plants are frequently specific depending on the type of herbivore and its age, abundance and feeding guild. The sensory perception of plant volatiles by herbivore enemies is also specific, according to the latest evidence from studies of insect olfaction. Thus, enemies do exploit the detailed information provided by plant volatile mixtures in searching for their prey or hosts, but this varies with the diet breadth of the enemy (MCCORMICK *et al.,* 2012).The volatile blends released by grasses in response to herbivory vary greatly in quantity and composition. In a sample of 32 maize lines, release rates from 0.7 to 54.2µg/hr/gm leaf dry weight were observed and suggest that some maize varieties are much more capable of attracting herbivore enemies than others (DEGEN *et al*., 2004). The high variation between volatile blends argues against a single volatile signal that is common to grasses. Especially in maize, the variation in volatiles between different genotypes appears too large to be perceived as a specific signal. However, *C. marginiventris*, like most other parasitic wasps, can associate a successful oviposition experience with the volatiles encountered at that time (TURLINGS *et al*., 1990). Therefore, the parasitoid locates its hosts by both innate responses and associative learning of volatiles (HOBALLAH and TURLINGS, 2005). This allows the parasitoid to adapt and optimize its host-finding strategy toward the most rewarding plant signals.

In field experiments, parasitic wasps even show cross-recognition between different grass species. Intercropping maize with the molasses grass, *Melinis minutiflora*, significantly increased larval parasitism of stem borers by *Cotesia sesamiae* and decreased levels of infestation by stem borers in the crop (KHAN *et al*., 1997). This interaction is thought to occur since volatile components released by intact *M. minutiflora* are similar to those produced by herbivore-damaged maize plants (KHAN *et al*., 1997). The yellow stem borer, *Scirpophaga incertulas* Walker (YSB), infested rice plants emit chemicals through the surface of their infested stems. These induce attractant activity and cause arrestment responses and ovipositional stimulation in its egg parasitoid, *Trichogramma japonicum* Ashmead. Herbivore-induced plant chemicals are released through the stem surfaces and attract *T. japonicum*, even over long distances. These cues elicit parasitoid arrestment on pest damaged plants and subsequently lead to the successful parasitization of the stem borer (USHARANI and SANDHYARANI, 2012).

VOLATILE COMMUNICATION AMONG INFESTED PLANTS (PRIMING)

Another feature of the interesting and complex interplay between insects and plants is that plants can detect and react to chemical signals from neighboring plants. Chemical signals from a plant damaged by insect herbivores alert neighbors to prime their defenses so as to respond more strongly to subsequent attack than if they had not been forewarned. Thus, corn seedlings previously exposed to ‘green leaf’ volatiles (6-carbon aldehydes, alcohols, and esters) from damaged corn seedlings emitted at least twice the quantity of volatile terpenes when attacked by caterpillars as seedlings that had not been exposed to the ‘alarm’ signals (ENGELBERTH *et al.* 2004).

INTERACTIONS BETWEEN HERBIVORES AND PLANT PATHOGENS/ VIRUSES

Plant resistance mechanisms also affect plant quality in future interactions with attackers. LAZEBNIK *et al* (2014) proposed hypothesis that (i) biotrophic pathogens can facilitate chewing herbivores, unless plants exhibit effector-triggered immunity, but (ii) facilitate orinhibit phloem feeders. (iii) Necrotrophic pathogens, on the other hand, can inhibit both phloem feeders and chewers. They also propose herbivore feeding mode as predictor of effects on pathogens of different trophic strategies, providing evidence for the hypotheses that (iv) phloem feeders inhibit pathogen attack by increasing SA induction, whereas (v) chewing herbivorestend not to affect necrotrophic pathogens, while they may either inhibit or facilitate biotrophic pathogens.

It is well known that insects induce or suppress plant volatile compounds in such a way that they benefit from such plant alteration (KORADA *et al* 2013b). *Myzus persicae* and *B*. *tabaci* have similar roles in vectoring sweet potato viruses as both insects suppress the same types of volatile compounds (KORADA *et al.,* 2013b). Volatile emissions from healthy sweet potato plants (var. Beauregard), SPFMV infected plants, and healthy plants infested by two aphid species, *M. persicae* and *Aphis gossypii*, and the whitefly *B. tabaci* were analyzed. Healthy sweet potato plants (var Beauregard) emitted 22 volatile compounds (Fig.1). Colony forming aphid *M. persicae* suppressed 10 volatile compounds (1,2-dimethyl benzene, (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-decenal, sabinene, para-diethyl benzene, methyl salicylate, α-gurjunene, and trans-caryophyllene, etc.); whereas, *A. gossypii*, which does not colonize sweet potato, suppressed only 7 compounds. The whitefly, *B. tabaci*, successfully suppressed the same 9 volatile compounds that *M. persicae* did, except (*E*)-2-decenal. Furthermore, Sweet Potato Feathery Mottle Virus (SPFMV) infected plants (un-infested by any of the test insect) produced two extra volatile compounds, 2-hexenyl acetate and cyperene, which were not seen in healthy plants or vector-induced plants. Hence, the success and establishment of the SPFMV in sweet potato may be partly attributed to the production of 2-hexenyl acetate and cyperene by SPFMV infected plants (KORADA *et al.,* 2013b).

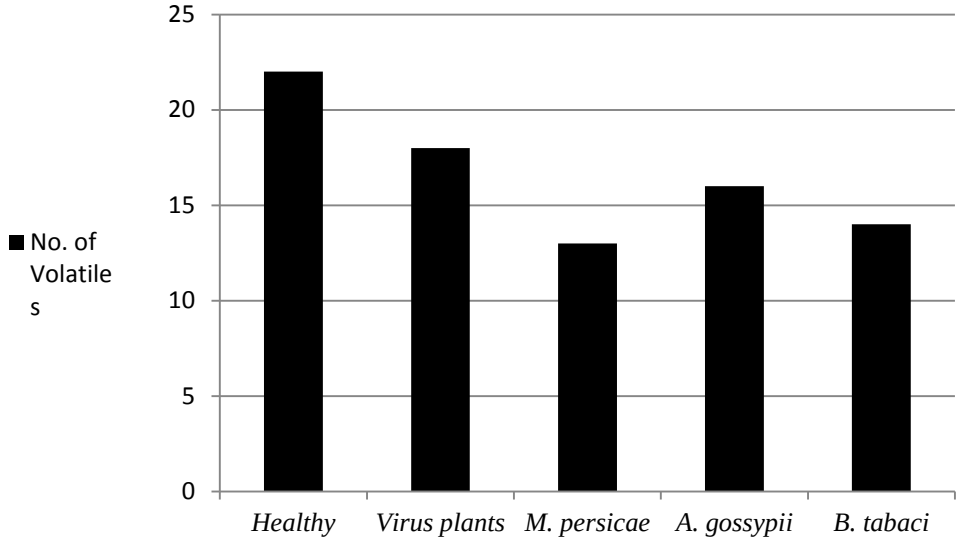

Fig. 1: Differences in volatile compounds emitted by sweet potato plants infected and uninfected by Sweet Potato Feathery Mottle Virus (SPFMV and induced by sucking pests

INTERACTIONS BETWEEN HERBIVORES AND INSECT-MICROBIOMES

Insect-associated microbes can influence host-plant detection and/or signaling through phytohormone synthesis, conserved microbial patterns, and effectors, however, microbes associated with insects must be maintained in the environment and located in opportunistic positions (CASTEEL and HANSEN, 2014). Insect herbivores are not alone, but in fact harbor various viruses, fungi, and bacteria in their bodies, guts, saliva, and/or on the surface of their exoskeleton. Plants too are colonized by numerous microbes both above and below ground, and in their phyllosphere and rhizosphere, respectively.

Environmental and gut microbes

Herbivores possess diverse microbes in their digestive systems, and these gut microbes can modify plant–insect interactions. For example, during host-plant feeding, the Colorado potato beetle (*Leptinotarsa decemlineata*) introduces environmental/gut bacteria into the plant, inducing transcripts related to JA and SA signaling in tomato (CHUNG *et al*., 2013). When beetles are cured of bacteria using antibiotics, transcripts related to JA signaling are strongly induced compared to feeding by untreated beetles. Bacteria introduced into the plant in isolation of insects, elicit marker genes of SA signaling and inhibit JA related transcripts and defense responses (CHUNG *et al.,* 2013). This suggests that JA induction by beetle feeding is suppressed when they secrete their environmental/gut microbes into the host-plant. Additionally, re-introducing the bacteria to antibiotic-treated larvae restores the insect's ability to suppress defenses (CHUNG *et al*., 2013). As SA signaling often inhibits jasmonate signaling, larvae may exploit bacteria in their oral secretions and suppress plant defenses.

Intra/Extracellular Microbes

One of the best examples illustrating the effect of insect microbes on plant signaling and metabolism was conducted on *Arabidopsis thaliana* and the leafhopper *Macrosteles quadrilineatus*, which vectors Aster Yellows phytoplasma, strain Witches' Broom (AY-WB) (SUGIO *et al*. 2011). AY-WB is obtained by the leafhopper during feeding on infected-plants. After an incubation period in the insect's body, AY-WB moves to the salivary gland where it can be injected back into a host plant during feeding. Leafhoppers feeding on AY-WB-infected plants have higher fecundity compared to uninfected plants, primarily due to the phytoplasma's ability to inhibit JA. Further, SUGIO *et al*. (2011) found that the phytoplasma's effector protein (SAP11) is responsible for JA inhibition, by destabilizing two Arabidopsis transcription factors.

HERBIVORE EFFECTORS (SUPPRESSOR OF VOLATILES)

There has been very limited work on identifying herbivore effectors, although the ability of herbivores to evade host defenses is becoming better appreciated. Insect herbivores produce a cocktail of effectors that can suppress plant defensive pathways, mimic plant hormones, and/or mask the perception of HAMPs. While the labial saliva of several noctuid species has been shown to suppress direct and indirect plant defenses, glucose oxidase (GOX) remains as the one identified salivary constituent contributing to this suppression. This enzyme, produced by the labial and mandibular salivary glands, oxidizes b-D glucose to form gluconic acid and H_2O_2. Secretion and synthesis of GOX is highly dependent upon the host plant and diet; indicating that the effects of the enzyme on plant defenses is likely to be context-dependent as described for HAMPs. Recent evidence indicates that in addition to suppressing direct defenses such as the induction of nicotine in tobacco, saliva (and perhaps GOX) can suppress the induction of volatile, indirect defenses.

MECHANISMS OF HERBIVORE-INDUCED VOLATILE PRODUCTION

Jasmonic acid triggered immunity (JATI)

The plant hormone jasmonic acid (JA) exerts direct control over the production of chemical defense compounds that confer resistance to a remarkable spectrum of plant associated organisms, ranging from microbial pathogens to vertebrate herbivores. The underlying mechanism of JA-triggered immunity (JATI) can be conceptualized as a multistage signal transduction cascade involving: i) pattern recognition receptors (PRRs) that couple the perception of danger signals to rapid synthesis of bioactive JA; ii) an evolutionarily conserved JA-signaling module that links fluctuating JA levels to changes in the abundance of transcriptional repressor proteins; and iii) activation (de-repression) of transcription factors that orchestrate the expression of myriad chemical and morphological defense traits. Multiple negative feedback loops act in concert to restrain the duration and amplitude of defense responses, presumably to mitigate potential fitness costs of JATI (CAMPOS *et al*., 2014).

Pattern-triggered immunity (PTI) confers basal resistance and is mediated by cell surface-localized pattern recognition receptors (PRRs) that bind conserved foreign molecules, known collectively as microbial/pathogen-associated molecular patterns (MAMPs). A second layer of induced resistance, referred to as effector-triggered immunity (ETI), relies on polymorphicintracellular resistance (R) proteins to detect effector molecules that plant attackers deliver into host cells to counteract defense.

In addition to cell surveillance systems that recognize foreign threats in the form of MAMPs/HAMPs and effectors, it has long been known that plant-derived (i.e., self) signals also are potent elicitors of local and systemic defense responses (MOUSAVI *et al.* 2013). These endogenous elicitors are produced in response to general cellular injury and may be classified as damage-associated molecular patterns (DAMPs). Because DAMPs are generated in response to diverse types of tissue injury, their role in cellular recognition of pathogen attack traditionally has been ignored. However, the recent identification of DAMP receptors and associated signal transduction components (CHOI *et al*., 2014; MOUSAVI *et al*., 2013) is shaping a broader view of how plant cells perceive and respond to injurious threats. The extent to which cellular recognition of a given threat is translated into a host response that specifically neutralizes the attacking pathogen or herbivore has also has to be understood. Indeed, genome wide transcriptome studies indicate a significant degree of overlap in molecular responses triggered by different MAMPs/HAMPs/DAMPs and effectors (CAILLAUD *et al*., 2013; GOUHIER-DARIMONT *et al.,* 2013; Kim *et al*., 2014; ZHUROV *et al.,* 2014). There is evidence to indicate that PTI and ETI converge on similar downstream signaling components, including MAPkinase pathways, ROS production, and calcium-dependent signaling events (ROMEIS and HERDE, 2014).

The central role of JA as an activating signal for induced immunity is grounded in three general observations. First, biotic attack and other forms of tissue injury result in the rapid synthesis of JA and its receptor-active derivative, jasmonoyl-L-isoleucine (JA-Ile). Stress-induced accumulation of JA-Ileoccurs in both above- and below-ground tissues and, dependingon the eliciting signal and tissue type, is a systemic response (MOUSAVI *et al*., 2013). Second, JA promotes the expression of virtually all major classes of secondary metabolites and proteins that have established roles in defense, including alkaloids, terpenoids, phenylpropanoids, amino acid derivatives, anti-nutritional proteins, and some pathogenesis-related (PR)proteins (DE VLEESSCHAUWER *et al.,* 2013). The JA pathway also promotes the development of morphological structures, including

glandular trichomes, resin ducts, and nectaries that produce a rich variety of compounds serving direct and indirect roles in defense. Studies employing JA mutants have demonstrated the crucial role of this hormone in plant protection against diverse biota. Among the plant-associated organisms whose fitness is curtailed by JATI are necrotrophic and (hemi)biotrophic pathogens, mutualistic fungi, nematodes, leafhoppers, beetles, caterpillars, aphids, thrips, spider mites, fungus gnats, slugs, crustaceans, and some vertebrate herbivores. Indeed, it is reasonable to think that the number of plant-eating species affected by JATI may exceed the total number of plant species on Earth.

Ca2+ signaling, ROS and MAPKs

JA synthesis is initiated in the plastid by stress-induced activation of lipases that release fatty acid precursors of JA(WASTERNACK and HAUSE, 2013). Alternatively, there is evidence to suggest that tissue damage may stimulate JA synthesis from an existing pool of OPDA. Among the intracellular signals implicated in this process are calcium ions, reactive oxygen species (ROS), and mitogen-activated protein (MAP)kinase cascades.

Calcium ions have long been recognized as ubiquitous second messengers in signal transduction pathways. The involvement of Ca2+ in JATI is supported by studies showing that cytosolic Ca2+ levels increase in response to herbivore feeding and treatment with exogenous MAMP/HAMP/DAMPs. Changes in membrane polarization caused by wounding and insect attack also increase the level of cytosolic Ca2+ (MAFFEI *et al.,* 2006). Ca2+ fluxes and associatedCa2+-binding proteins, including calmodulin and Ca2+-dependentproteinkinases (CDPKs), exert control during the activation of JA-response genes (ROMEIS and HERDE, 2014; YANG *et al*., 2012a). Dynamic changes in cytosolic Ca2+levels during plant-attacker interactions are linked to the production of ROS, including hydrogen peroxide (ARIMURA and MAFFEI, 2010). Studies in Arabidopsis, for example, show that a signal generated at the site of leaf injury travels rapidly (2–3 cm/min) to trigger JA-I synthesis and associated JA responses in undamaged leaves.

Manipulation of JATI by herbivores

An important emerging paradigm in plant-herbivore interactions is the ability of herbivores to activate the SA pathway and thereby reduce the effectiveness of JATI as a basal defense (HOGENHOUT and BOS, 2011). For example, phloem feeding by silver leaf whitefly (*Bemisia tabaci*) results in increased expression of SA-related defense genes and concomitant repression of JATI (Zhang *et al*.2013). Similarly, insect egg-associated effectors trigger SA-accumulation and JATI suppression in host tissues surrounding the egg, thus favoring the survival of newly hatched larvae (REYMOND, 2013).

Secretion of SA into the locomotion mucus (slime trail) by some molluskan herbivores KÄSTNER *et al*. (2014), or excretion of SA into honeydew by some aphid species (SCHWARTZBERG and TUMLINSON, 2013), may reflect additional mechanisms to suppress JATI. The Coleopteran herbivore *Leptinotarsa decemlineata* (Colorado potato beetle) employs an alternative but no less effective strategy to hijack JATI; symbiotic bacteria in the oral secretion of the beetle activate SA-dependent responses and repress local and systemic JATI (CHUNG *et al.,* 2013). That this phenomenon also occurs in a root-feeding insect herbivore (*Diabrotica virgifera*, western corn rootworm) of maize suggests that host defense suppression by symbiotic bacteria maybe a general feeding strategy adopted by insect herbivores (BARR *et al*., 2010).

CONCLUSIONS

The defensive reactions of the plant, following physical injury by the herbivore, are influenced by a multitude of factors including, but not necessarily limited to, the elicitors and numerous other herbivore-associated molecules, as well as microbes on the plant surface that may alter plant defensive pathways. Ultimately, a thorough and accurate understanding of the chemical ecology of insect–plant interactions will require a more holistic approach, taking into consideration the ecological and physiological context in which a plant perceives and responds to herbivore-associated signals.

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OCCURRENCE OF ANTIBIOTIC-RESISTANT *ENTEROCOCCI* IN SOME INSECTS FROM STORED FOOD PRODUCTS IN BOTSWANA

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ABSTRACT: The occurrence of antibiotic-resistant enterococci in some insects from stored food products from fourteen geographical areas in Botswana was investigated in the present study. Eleven insect species were identified from a total of 737 stored food products. *Oryzaephilus surinamensis* was the most predominant. It comprised 65% and 51.1% of the speciesin food products from households and the selected warehouse respectively; followed by *Tribolium castaneum*, which comprised 17.1% of the species in stored foods from households and 32.3% of the species in stored food products from the warehouse. There was no significant difference ($p<0.05$) in the distribution of the insect species from the geographical areas sampled.Sixty-eight per cent (68%) of the total insect numbers harboured *Enterococcus* species. *Enterococcus* species were isolated from *Prostephanus truncatus* and C*ryptolestes ferrugeneus*, obtained from laboratory stored maize cultures. The isolation rate of enterococci was significantly higher ($p<0.05$) in laboratory stored product insects as compared with insects collected from stored foods sampled from the selected geographical areas. Enterococci showed total resistance to erythromycin, fusidic acid, oxacillin, novobiocin, and penicillin G but were all sensitive to chloramphenicol. This shows the capacity of some stored product insects to carry antibiotic-resistant and potentially pathogenic *Enterococcus* species. The latter is of public health importance and therefore there is need to monitor the occurrence of *Enterococcus* species in insects infesting stored products. These insects can act as vectors of pathogenic microorganisms & cause health hazards to consumers if proper pest management strategies are not applied to stored products.

Key words: *Oryzaephilus surinamensis, Prostephanus truncatus, Cryptolestes ferrugineus, Tribolium castaneum, Enterococcus* species, Enterococci, antibiotic resistance

INTRODUCTION

Cereals and pulses are important food staples in Botswana and represent significant portions of the daily dietary intake (ALLOTEY, 1991; ALLOTEY and RAMONGALO, 2011). Insect species such as *Sitotroga cerealella* (Olivier), *Ephestia cautella* (Walk.), *Callosobruchus maculatus* (Fabr.), *Rhyzopertha dominica* (Fabr.), *Sitophilus zeamais* Motsch, *Tribolium castaneum* (Herbst.), and *Tribolium confusum* (J. du Val.) have been reported in food commodities in Botswana (ALLOTEY *et al*., 2010; MOHALE *et al*., 2010; ALLOTEY *et al*., 2011; ALLOTEY, 1991; ALLOTEY and MOLOKO, 2015). They cause significant food losses during storage (ALLOTEY *et al*., 2010; ALLOTEY and MOLOKO, 2015) and their control is of paramount importance to food security (ONIANG'O and ALLOTEY, 1999). Some stored products insects are known to harbour pathogenic and potentially pathogenic bacteria (CHANNAIAH *et al*., 2010). *Sitophilus oryzae* (L.) has been reported to carry *Salmonella* sp. (ALLOTEY, 2011; BLAZAR *et al*., 2011). *Staphylococcus* and *Bacillus* species have been isolated from the gut of *Callosobruchus maculatus* (SEVIM *et al*., 2016).CHANNAIAH *et al*. (2010) have addressed the importance of stored product insects carrying enterococci.

Enterococci have previously been isolated from some perishable food products such as milk, chicken, beef and beverages in Botswana (COLLISION and JOHANNES, 1999; CHINGWARU *et al*., 2003; AAKU *et al*., 2004; MATSHEKA *et al*., 2013). Prior to the present study there has been no report on enterococci in stored product insects in Botswana. *Enterococcus* species are Gram positive cocci and occur in diverse environments such as soil, animal food products, gastrointestinal tracts of animals and animal excreta (FRANZ *et al*., 1999). These bacterial species are established pathogens especially incausing hospital-acquired infections (LINDEN *et al*., 1999) and are also opportunistic pathogens in case of immune-suppression such as HIV/AIDS infection (AWANDA *et al*., 1999). They also cause infectious diseases such as bacteraemia, urinary tract infections, endocarditis, wound and tissue infections (KAYSER, 2003). Enterococci are known for their resistance to a wide array of antibiotics (MURRAY, 1990) and they are also specifically recognized as important reservoirs of antibiotic resistance genes that can spread horizontally to other bacterial pathogens (DEVRIESE *et al.*, 1992). The objectives of the present study were to identify insects associated with some stored products and to determine their potential of carriage of antibiotic-resistant enterococci.

MATERIALS AND METHODS

Collection of insects: Insect infested foods were collected from households in 12 different places, mostly from the southern part of Botswana, the Botswana Agricultural Marketing Board (BAMB) warehouse in Gaborone and from the Insectary in the Department of Biological Sciences, University of Botswana. Localities sampled were mostly from southern Botswana (Gaborone, Mochudi, Otse, Lobatse, Molepolole, Tlokweng, Kopong, Good hope and Pitsane) and northern Botswana (Mahalapye and Dibete). Insect-infested food products were sifted using standard sieves (Humboldt, Chicago, Illinois, USA) to obtain live insects. The live insects were weighed and thereafter insect species were identified. The present study was conducted from November 2014 to May 2015.

Isolation and identification of enterococci: Each identified insect was surface sterilized using 10% sodium hypochlorite in a Petri dish and further sterilized by immersion into 70% ethanol. The surface sterilized insect was homogenized in 1ml phosphate buffered saline. The homogenate (5µl) was then drop plated onto mEnterococcus agar (HiMedia, Bangkok, India) and the agar plates incubated at 37^0C for 24h.To identify enterococci, red and pink colonies on mEnterococcus agar (characteristic of *Enterococcus* species) were Gram stained and the catalase test performed. Gram positive and catalase negative cocci were tested for β-hemolysis on blood agar and β-hemolytic colonies were presumed to be *Enterococcus* sp. To confirm these isolates as enterococci, the bile esculin test, growth in 6.5% NaCl and L-pyrrolindonyl-β-naphthlyamide (PYR) tests were performed. Isolates that tested positive for all the three tests were confirmed as *Enterococcus* sp. The isolates were not identified to the species level.

Antimicrobial susceptibility testing: To perform antibiotic susceptibility, the test isolates were standardized to 0.5 McFarlands in 0.1% physiological saline. Using a swab, the bacterial suspension was spread throughout Mueller Hinton agar (Oxoid, Basingstoke, UK) plates before performing antimicrobial susceptibility testing for all the positive isolates using the disc diffusion test. All the antibiotics were obtained from Mast Group (Merseyside, UK). The eight antibiotics used were: Chloramphenicol (25µg), Erythromycin (5µg), Fusidic acid (10µg), Oxacillin (5µg), Novobiocin (5µg), Penicillin G (1U), Streptomycin (10µg) and Tetracycline (25µg). Plates were then incubated at 37°C for 24h. After incubation diameters of zones of clearing around colonies were measured

and recorded and the results were interpreted using the criteria from the Clinical & Laboratory Standards Institute (2006).

The data was analysed using Statistical Package for Social Science (IBM SPSS 21.0). Pearson correlation was employed to demonstrate relationship between sampling locations, food commodity type, identified insect species and percentage of isolates that were positive for enterococci. One-way ANOVA was utilized to separate the means of the percentage of the positive enterococci isolates according to the zones sampled and insect species.

RESULTS AND DISCUSSION

Oryzaephilus surinamensis was the predominant insect species found in food samples from Extension 4, Goodhope, Pitsane, Mochudi, Otse, Broadhust and Mahalapye (65.8% of locations had *O. surinamensis*). Out of the 8 infested food samples from the warehouse, 6 had *O. surinamensis* which made it the most common insect species (Table-1). All households collected foods had one insect species except the farinaceous soup product from Mochudi which had two species, *Tribolium castaneum* and *O. surinamensis.*Insects from household food samples constituted 35.2% of the total insect population recorded, while those from the warehouse constituted 18.6%.

Ten (10) infested food commodities from the insectary were utilized for this study. *Prostephanus truncatus* and *Cryptolestes ferrugineus*from maize were the dominant species constituting 28.6% and 24.2%, respectively (Table-2). However, the food commodity did not have any statistical significance on the identified insect species ($p<0.05$; $p=0.000$). Only 1 (0.3%) *Anthrenus* sp. was found from biltong. Maize had multiple infestations of 3 species *viz*., *P. truncatus, Rhyzopertha dominica* and *C. ferrugineus* whereas most food commodities had one species.

P. truncatus and *C. ferrugineus* were the two insect species that were positive for enterococci, both from the insectary stored maize (Table-3). Of the 98 *P. truncatus* and 83 *C. ferrugineus*, 34% and 20% were positive for *Enterococcus* sp., respectively. Overall, the occurrence of enterococci in the two species was significantly higher than that of other insect species, with 95% confidence ($p<0.05$; $p=0.000$). The two insect species positive for enterococci constituted 6.8% of the total live insects which were identified. All the enterococci isolates from the two insect species were resistant to novobiocin, methicillin, erythromycin, fusidic acid, and penicillin G but susceptible to chloramphenicol. Ninety (90) per cent and 30% of the isolates were resistant to tetracycline and streptomycin, respectively whereas 10% and 70% of the isolates were susceptible to tetracycline and streptomycin, respectively (Table-4). None of the enterococci isolates was intermediate to any antibiotic.

From the above results, the most predominant insect species associated with stored products in this study was *O. surinamensis* which comprised of 65.8% and 51.1% of species in the households and the Botswana Agricultural Marketing Board (BAMB) warehouse, respectively. *Tribolium castaneum* was the second predominant insect species in both the warehouse (32.3%) and households (17.1%).

Table-1: Insect species identified from various stored-product foods collected from households and a warehouse in Botswana

Sampling location	Stored-Product sample (N_h=23, N_w=8)*	Insect species identified	No. of insects (%)
Households (h)			
Goodhope	Sorghum	*Oryzaephilus surinamensis*	12 (4.7)#
Lobatse	Samp	*Tribolium confusum*	2 (0.8)
	Flour	*Tribolium castaneum*	8 (3.1)
Gaborone (Extension 4)	Sorghum	*O. surinamensis*	32 (12.5)
	Samp	*T. castaneum*	2 (0.8)
Pitsane	Sorghum	*O. surinamensis*	24 (9.3)
Dibete	Samp	*T. castaneum*	13 (5.1)
	Cowpeas	*Callosobruchus maculatus*	6 (2.3)
Mochudi	Samp and beans combination	*T. castaneum*	9 (3.5)
	Farinaceous soup	*T. castaneum*	5 (1.9)
		O. surinamensis	28 (10.9)
	Sorghum	*O. surinamensis*	16 (6.2)
Otse	Wheat	*T. castaneum*	7 (2.7)
	Rice	*O. surinamensis*	14 (5.4)
	Sorghum	*O. surinamensis*	8 (3.1)
Gaborone (Broadhurst)	Whole maize	*Sitophilus zeamais*	2 (0.8)
	Crushed maize	*O. surinamensis*	12 (4.7)
	Green peas	*C. maculatus*	9 (3.5)
Tlokweng	Maize meal	*T. confusum*	6 (2.3)
Molepolole	Black eyed peas	*C. maculatus*	4 (1.6)
Kopong	Haricot beans	*C. maculatus*	15 (5.8)
Mahalapye	Sorghum	*O. surinamensis*	16 (6.2)
	Maize meal	*O. surinamensis*	7 (2.7)
Total (T_h)			**257 (34.9)**
Warehouse (w)	Maize rice	*O. surinamensis*	3 (2.2)
		T. castaneum	1 (0.7)
	Samp	*O. surinamensis*	12 (8.8)
		T. confusum	8 (5.8)
	Rice	*O. surinamensis*	16 (11.7)
		T. castaneum	3 (2.2)
	Cowpeas	*C. maculatus*	13 (9.5)
	Homemade brew	*T. castaneum*	31 (22.6)
		O. surinamensis	21 (15.3)
	Flour	*T. castaneum*	4 (2.9)
	Maize meal	*T. castaneum*	6 (4.4)
		O. surinamensis	12 (8.8)
	Sorghum	*O. surinamensis*	7 (5.1)
Total (T_w)			**137 (18.6)**

*N_h= No. of insect infested food from households; N_w= No. of insect infested food from the warehouse; T_h= Total number of insects from households; T_w= Total number of insects from the warehouse; #Numbers in parentheses indicate percentages.

Table-2: Infestation of stored products by insects in the insectary at University of Botswana

Food commodity	Insect species	Number (%) of insects
Biltong	*Lasioderma serricorne*	10 (2.9)[#]
	Anthrenus sp.	1 (0.3)
Phane	*Stegobium paniceum*	23 (6.7)
Maize	*Prostephanus truncatus*	98 (28.6)
	Rhyzopertha dominica	12 (3.5)
	Cryptolestes ferrugineus	83 (24.2)
Cowpeas	*Callosobruchus maculatus*	17 (5.0)
Maize meal	*Tribolium castaneum*	21 (6.1)
	Tribolium confusum	6 (1.7)
Rabbit feed	*Sitophilus zeamais*	2 (0.6)
Flour	*Tribolium confusum*	18 (5.2)
	T.castaneum	4 (1.2)
Spices	*T.castaneum*	9 (2.6)
Sorghum	*Oryzaephilus surinamensis*	32 (9.3)
Bambara nuts	*C. maculatus*	7 (2.0)
Total		**343 (46.5)**

[#]Numbers in parentheses indicate percentages.

Table-3: Prevalence of enterococci in stored product insects isolated in three localities

Insect species isolated from:	Total no. of insects	No. (%) of isolates positive for enterococci
Households and Warehouse		
1. *O. surinamensis*	240	0 (0)[#]
2. *C. maculatus*	38	0 (0)
3. *T. castaneum*	96	0 (0)
4. *T. confusum*	16	0 (0)
Insectary		
1. *L. serricorne*	10	0 (0)
2. *S.paniceum*	23	0 (0)
3. *P. truncatus*	98	33 (33.6)
4. *C. ferrugineus*	83	17 (20.5)
5. *O. surinamensis*	32	0 (0)
6. *C. maculatus*	17	0 (0)
7. *T. castaneum*	34	0 (0)
8. *T. confusum*	18	0 (0)
9. *S. zeamais*	2	0 (0)
10. *R. dominica*	12	0 (0)
11. *Anthrenus* sp.	1	0 (0)
Total	**730**	**50 (6.8)**

[#]Numbers in parentheses indicate percentages

These results are in agreement with a previous study by LARSON *et al*. (2008), who reported *T. castaneum* to be the second predominant insect species in feed mills in the Midwestern United States. From the insectary *P. truncates* (29.1%) was the predominant species in maize followed by *Cryptolestes ferrugineus* (24.6%). These two species have been reported to be associated with maize infestation (BELL and WATTERS, 1982; WHITE *et al*., 1995).

Table-4: Antibiotic susceptibility of *Enterococcus* sp. isolated from *P. truncatus* and *C. ferrugineus*

Antibiotic	% Resistant	% Intermediate	% Susceptible
Novobiocin	100	0	0
Streptomycin	30	0	70
Methicillin	100	0	0
Erythromycin	100	0	0
Tetracycline	90	0	10
Penicillin G	100	0	0
Fusidic acid	100	0	0
Chloramphenicol	0	0	100

Notably, *P. truncatus* and *C. ferrugineus* were the only stored product insects from which antibiotic resistant *Enterococcus* sp. were isolated. The association of antibiotic resistant enterococci with maize is significant considering the fact that maize forms one of the main dietary staple foods in Botswana. It can be noted that enterococci were not isolated from stored product insects in households and the warehouse. Samples from the warehouse and households represent fresh harvest from the farm for immediate consumption whilst samples from the insectary are for teaching purposes and have been in some instances stored and handled for up to 10 years. It is therefore likely that the keeping time of samples in the two settings could have had a bearing on the differences in the occurrence of antibiotic resistant enterococci. This study profiles the occurrence of antibiotic resistant *Enterococcus* sp. in stored product insects in Botswana. Antibiotic resistance in enterococci to a wide range of antimicrobials is a significant cause of concern globally, and this resistance can either be acquired or intrinsic (KAYSER, 2003). Enterococci have been found to be resistant to β-lactams, cephalosporins, sulphonamids, chloramphenicol and vancomycin (KAYSER, 2003; RADU *et al*., 2001).

Enterococcus species in the present study displayed high resistance to antibiotics employed, and only showed complete sensitivity to chloramphenicol. Total resistance was encountered for erythromycin, fusidic acid, novobiocin, penicillin G and methicillin. Resistance to β-lactam antibiotiotics such as penicillin G and methicillin was not unexpected because enterococci have an intrinsic mechanism of resistance to these antimicrobials through the overproduction of the essential target, the low affinity penicillin binding protein 5 (PBB5), or mutations of different residues at its active site (ZORZI *et al*., 1996). It is highly probable that a significant portion of these resistant isolates represent vancomycin resistant enterococci (VRE) which have been implicated in 8% of nosocomial infections in the USA (CENTERS FOR DISEASE CONTROL AND PREVENTION, 1993). Of public health importance is the finding that VREs are capable of transferring antibiotic resistance genes horizontally to more serious human pathogens such as *Staphylococcus aureus* (FRANZ *et al*., 1999).

The reasons for the high antibiotic resistance of enterococci in this investigation are not clear, but resistance in these species has been linked to the use of antibiotics in feed as growth promoters (WITTE, 2000). It is important to note that in Botswana, there are no standard regulations on the use of antibiotics in feed and as a result antibiotic resistance due to this problem cannot be precluded. To confirm this association however, further studies are warranted. Enterococci are known indicators of food sanitary quality while their presence in insects is thought to be of animal fecal matter origin (JAY *et al*., 2005).The absence of enterococci in stored product insects in households as well as the warehouse may indicate good sanitary practices in these environments. The source of enterococci in the insectary could be a result of cross-contamination by personnel through extensive handling over a long time, especially where aseptic techniques were not enforced. Because the insect species detected in this study are highly mobile and capable of flight (e.g., *T. castaneum* and *P. truncatus*), one cannot rule out the possibility of cross-contamination of other stored food products with pathogenic antibiotic-resistant *Enterococcus* sp.

Thus the present study shows that stored product insects are capable of being important carriers of potentially pathogenic enterococci. Although stored food produce from the households and warehouse were not associated with enterococci in this study, strategic pest control measures and effective food management practices, including good sanitary practices and enacting regulations on the use of antibiotics in animal feeds can act as the panacea in reducing the dissemination of enterococci through insect pests/vectors in stored food products.

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LIFE CYCLE AND BEHAVIOUR OF *HESTIASULA BRUNNERIANA* AND *EUANTISSA PULCHRA* (ACROMANTINAE: HYMENOPODIDAE, MANTODEA) - PREDATORS IN CASHEW PLANTATIONS

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ABSTRACT: Praying mantids are one of the common predators in cashew plantations of South-West Karnataka and few species are seen throughout the year. The mantids feed on many cashew pests including tea mosquito bug, leaf beetles, leaf feeding caterpillars etc. Knowledge on ecology, life history, breeding, rearing feasibility and behavior of many of the mantid species is scanty. Our study aimed at recording the life cycle and behavior of two common mantid species in cashew plantations namely, *Hestiasula brunneriana* Saussure and *Euantissa pulchra* F. (Acromantinae: Hymenopodidae) under captive breeding using greater wax moth larvae as prey. In case of *H. brunneriana,* incubation period lasted for 26.65±0.27 days and there were seven nymphal instars, completed in 78-120 days. Whereas in *E. pulchra*, incubation period was just 9.38±0.18 days and the nymphal period comprised of six instars. Unlike *E. pulchra*, early instar nymphs of *H. brunneriana* managed well to feed on tiny wax moth larvae and survived well. Cannibalism was not observed during nymphal stages when reared in groups of 3 or 4 with sufficient prey. Though incubation period and nymphal developmental period were short in *E. pulchra*, adult longevity, oviposition period and fecundity were high compared to *H. brunneriana*. The period of sexual maturity, ovipositional behaviour and the courtship behaviour of the mantids were also recorded.

Key words: praying mantid, cannibalism, ootheca, wax moth larvae; mating, behavior

INTRODUCTION

The cashew (*Anacardium occidentale* L., Anacardiaceae) is a commercial tree nut crop known to be infested by more than 150 insect pests. Among the pests, tea mosquito bug and cashew stem and root borers are considered as chief pests in majority of cashew growing regions, while leaf miners, leaf and blossom webbers, apple and nut borers and thrips are the other important pests. Up to 40-50% yield loss has been reported due to insect pest damage in few cashew growing regions (SUNDARARAJU and SUNDARABABU, 1999, ASOGWA *et al*., 2008, DWOMOH *et al*., 2012). Chemical management is widely adopted for pest control in cashew. Research insights in to ecofriendly pest management methods especially biological pest control in cashew are necessary in the changing pest management scenario. Like spiders and reduviids (BHAT *et al*., 2013 a, b), mantids are also common predators in the cashew plantations. The knowledge on bio ecology, behaviour and pest suppression efficacy of any organism is very essential to use it as a successful biological control agent. Mantids are considered as difficult group of insects for rearing, hence only few biological aspects have been documented on mantid species. During the present investigation, attempts have been made to record the life cycle and breeding behaviour of two common mantid species, namely, Indian unicorn boxer mantis, *Hestiasula brunneriana* and ant mimicking mantids, *Euantissa pulchra* (Acromantinae: Hymenopodidae, Mantodea) which are the common mantid species in cashew plantations of study area. These mantid species remain active

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during flushing and flowering period of cashew and found predated upon many cashew insect pests including tea mosquito bug, leaf weevils, ants, grasshoppers, leaf beetles, small caterpillars, hoppers etc during August- March. The present investigation aimed to understand the biology, behaviour and rearing feasibility of these two mantids under laboratory conditions using greater wax moth larvae so as to find out possible ways to utilize them in biological control programmes of cashew pests.

MATERIALS AND METHODS

a. Collection and initial culture of mantids

Surveys were done in random locations of the cashew plantations at fortnight intervals during 2012 – 2015 in 60 ha area at Puttur, Karnataka located in West coast of India (12.45° N Latitude, 75.4° E Longitude and 90 m above MSL) to study the occurrence pattern of the praying mantids. All-out-reach method was followed to find and collect the stages of praying mantids from the cashew plantations including tree base, litter, trunk, canopy of cashew trees, common weed plants as well as wire fences around the plantations. During the survey, *H. brunneriana* and *E. pulchra* were commonly noticed in the cashew plantations, and also on few weed species. Female mantids of both species were collected from the cashew plantations and brought to the laboratory to be maintained as breeding stock. The mantids were transferred individually into separate transparent cylindrical glass bottles of 500 ml capacity covered with muslin cloth and small dry cashew sticks were provided as perch. The prey insects used were larvae of greater wax moth (*Galleria mellonella*) maintained hygienically in lab and cultured in the standard artificial diet (PDBC, 2007). Each mantid was provided with 1-2 healthy fourth or fifth instar wax moth larvae daily and observed regularly for any oothecae laid. Care to be taken to avoid more larvae of wax moth which may result in webbing and mantids may get tangled in the web. Once ootheca was laid, the adults were separated and released into similar fresh glass bottles for further egg laying. The nymphs emerged were reared for further studies.

b. Rearing of mantids

Biology: Biology of mantids was studied in the laboratory following the methodology adopted by VANITHA *et al*. (2016). On the day of hatching, the 1st instar nymphs (hatchlings) were provided with minute water droplets sprinkled on the sides of the hatching glass bottles, and tiny larvae of greater wax moth were provided as prey. Next day, active nymphs were transferred individually into clean test tubes (2.5 cm dia. x 15 cm length) using a fine camel hair brush and 1-2 wax moth larvae (5-6 mm) were provided as prey and the tubes were plugged with sterile cotton. Subsequently, as the nymphs grew, bigger sized larvae were provided. Exuviae were removed after each moult and different stadial periods were recorded sequentially. Mantids were shifted into new test tubes once in a week to maintain cleanliness. The nymphs that grew more than 1.5 cm were reared individually in cylindrical glass bottles (500 ml capacity), so as to allow them to moult freely as adults, and the emerging male and female mantids were maintained separately (Fig 1a).

Sexual behavior: The mating arena consisted of a cylindrical transparent glass bottle of 2.5 litre volume (20 cm height x 12 cm dia.) covered at top with muslin cloth, having dried cashew sticks and few leaves at bottom to serve as shelter as well as hiding place for mantids especially male as adopted by VANITHA *et al*. (2016). Initially, four fully grown wax moth larvae (1.5-2.0 cm) were released into the mating arena and a female was introduced ten minutes before introducing a male. Care was taken not to release the male, when the female was hungry. Second day onwards, two to three wax moth larvae were released daily throughout the observation period. Five such pairs (N=5 pairs) were

observed for the sequence of sexual behaviour. The oothecae laid were removed at regular intervals and maintained in separate glass bottles to record the fecundity and hatchability. All the biological parameters were recorded sequentially, analysed and expressed as mean ± SE. T test was carried out to find any significant difference between male and female lifecycle events of each species.

RESULTS AND DISCUSSION

Seasonality: During surveillance in the cashew plantations, different stages of *H. brunneriana* were noticed throughout the year, and more numbers were sighted during August to February. Nymphs were seen during March to November, and adults during June to April, indicating existence of overlapping generations. The nymphs were also noticed on weed species in cashew plantations including *Getonia floribunda*, *Iroxa* sp. *Chromolaena odorata* and young plants of *Terminalia paniculata*. While, nymphs and adults of *E. pulchra* were noticed mid rainy season onwards (July to August) but frequently noticed during flushing and flowering period of cashew.

Biology: Laboratory rearing indicated the suitability of wax moth larvae for rearing both the mantid species. Freshly laid ootheca was white in *H. brunneriana* which turned into ivory white in a period of 3-4 days (Fig. 1a). Incubation period lasted for 26.15 ± 0.10 days (Table-1). Whereas in *E. pulchra*, oothecae were creamy in colour (Fig. 2a), and incubation period was just 9.38 ± 0.18 days (Table-2). Freshly hatched nymphs of *H. brunneriana* were dark chocolate brown with white patches on legs, while, the late instars develop white patches near thorax and abdomen (Fig. 1b). They fed well on small active greater wax moth larvae, and managed well even bigger larvae compared to their size. There were seven nymphal instars completed in 82-94 days, and the size of the nymphs gradually increased as they grew (Table-1).

Table-1: Developmental duration of *H. brunneriana* under laboratory conditions (X= 56)

S. No.	Life stages	Duration in days (mean ± SE)	
		Male	Female
1	Incubation period	26.15 ± 0.10	
2	Nymphal stages		
a	1st instar	11.25 ±0.89	11.62 ±0.88
b	2nd instar	14.5±2.10	14.62 ±1.03
c	3rd instar	9.08±0.71	10.00 ±0.76
d	4th instar	9.58±0.58	9.97 ±0.82
e	5th instar	12.67±0.75	13.15 ±0.46
f	6th instar	12.13±0.48	12.91 ±0.85
g	7th instar	18.46±1.56	18.79±1.33
3	Total nymphal developmental period	87.66±1.01	90.06±0.87
4	Nymphal survivability	85-95 %	
5	Adult longevity	75±0.64	88.75±0.76*
6	Total life cycle	158.66±3.29	179.81±2.54*

(*- significant at 0.05 % by t test, other values are non-significant).

Whereas, the early instars of *E. pulchra* were full black in colour resembling ants and the colour change occured in subsequent stages with development of brown, white and pinkish tinges on the body. Colour of the legs changed from black to brown and then to green (Fig. 2b, 2c and 2d). In the case of *E. pulchra,* the nymphs underwent six instars to

become adults in a period of 53-65 days (Table 2). In both mantids, wing buds started developing during fourth - fifth instars and became prominently visible during the final instar. Cannibalism was not noticed in both mantids at any of the nymphal stage, when 3 or 4 nymphs were kept together with sufficient prey. Upon disturbance, the nymphs of *H. brunneriana* exhibited characteristic threatening pose by spreading its forelegs. Nymphal survival was 85-95 % in *H. brunneriana*, whereas, it was only 10-20 % in *E. pulchra* under laboratory conditions (Tables 1, 2).

Table-2: Developmental duration of *E. pulchra* under laboratory conditions (X= 88)

S. No.	Life stages	Duration in days (mean ± SE)	
		Male	Female
1	Incubation period	9.38 ± 0.18	
2	Nymphal stages		
a	1st instar	11.83±0.62	11.85 ±0.51
b	2nd instar	7.09±0.16	7.25±0.18
c	3rd instar	8.67±0.22	8.92±0.15
d	4th instar	8.75±0.28	9.00±0.35
e	5th instar	10.08±0.26	10.17±0.24
f	6th instar	11.25±0.22	11.33±0.19
3	Total nymphal developmental period	57.08 ±0.98	58.50±0.60
4	Nymphal survivability	10-20 %	
5	Adult longevity	95.58±2.67	127.50±3.72*
6	Total life cycle	152.05±3.35	185.38±2.58*

(*significant at 0.05 % by t test, other values are non-significant).

Table-3: Ovipositional behaviour of *H. brunneriana* and *E. pulchra* under laboratory Conditions (X=8)

S. No.	Particulars	Duration/units	
		H. brunneriana	***E. pulchra***
1	Period of sexual maturity	13-15 days	9-13 days
2	Pre oviposition period	10-15 days	10-14 days
3	Days to first oviposition since emergence	27-32 days	12-20 days
4	Oviposition period	35- 40 days	102-118 days
5	Duration of oviposition/ootheca	35-45 min	40-50 min
6	Frequency of oviposition	6-14 days	7-10 days
7	Post oviposition period	13-17 days	6-18 days
8	Fecundity	5-7 oothecae	8-15 oothecae
9	Eggs/ootheca	8-16 nos	15-22 nos
10	Hatchability	85-90 %	92 %

Adult mantids of *H. brunneriana* exhibited characteristic death feigning by falling down sideways and remaining static for 0.5 - 1 minute. The female mantids were bigger (3±0.14 cm) compared to males (2.42 ±0.27 cm), stout, having bulky abdomen and shorter antennae with prominent unicorn at the head (Figure 1c). But, the males were slender, very active, smaller having longer antennae (1.3-1.5 cm). Wings extended beyond the abdomen in both sexes (Figure 1c). Sex ratio was male biased. Longevity of the female mantids was high, that lived 20-25 days longer than the male mantids. Whether mated or not, the female mantids started laying their first ootheca generally after 27-32 days of emergence, but, oothecae laid without copulation were infertile. Oviposition period was 35-30 days and oviposition interval was 6-14 days (Fig. 1d). A maximum fecundity was recorded as 7 oothecae/female. Number of eggs per ootheca ranged between 8 and 16 eggs and the hatchability was 85-90 % (Table-3). In the case of *E. pulchra* adults are green in colour (Fig. 2). Male mantids were tiny (1.93±0.08 cm) compared to female mantids (2.22±0.13 cm). In female, abdomen was stout and wings were slightly shorter exposing abdominal tip. Longevity of females was recorded up to 127.50±3.72 days, while that of males was only 95.58±2.67 days (Table-2). Sexual maturity was noticed in a period of 12-14 days. Female laid its first ootheca after 12-20 days of emergence with the fecundity of 8-15 oothecae/ female and the ovipositional frequency was 7-10 days (Table-3).

Sexual behavior: In both species, the male and the female mantids exhibited little interest in each other during the first week of emergence and initial days of second week. Sexual maturity was observed at the end of second week of adult emergence. Pre-mating cannibalism of the males occurred almost in all trials when they were kept together before sexual maturity. In both the cases, the male mantids attained sexual maturity 3-5 days earlier than the females. Sexual behaviour consisted of two distinct phases, *viz.*, preliminary courtship (1-10 min) and copulation (1-2 days). Upon noticing the female mantid, the male started moving around the mating arena and made short flights to attract the attention of the female. In most cases, the male slowly approached the female from its back, speeding up as it neared, and then mounted on the back of the female, thereby avoiding being caught and consumed by the female. In other rare courtship behaviour, there was a frontal approach by the male.

The courtship behaviour observed in both the mantids include the in sequential events *viz.,* speedy oscillation of the antennae by both the sexes, 'calling' of the receptive female by lowering the tip of the abdomen, and lifting of the wings slightly by female to expose the abdomen, an extremely slow and deliberate approach of the male towards the female, a quick flying leap by the male and climbing on the back of the female either frontal or from rear, antennal lashing of the male over the female, clasping the female thorax and wing bases with the forelegs, mounting and riding over, stretching and 'S-bending' of the terminal abdominal segments by the male, copulation, riding around by male on the back of the female (2- 14h) after copulation and finally quick flying away of the male. Duration of copulation was long in *H. brunneriana* (5.30–5.45h) compared to *E. pulchra* (35-50 min). Sexual cannibalism was not observed in both the mantids. In both species, multiple mating was seen in 2-3 days when male was left in the same container after courtship. After which, though there was sufficient prey, the male was definitely consumed by the female (but not at mating), during its repeated attempts to reach the female. Praying mantids are one of the predators in cashew plantations noticed throughout the year. Several species of mantids have been reported as effective predators of many defoliating insect pests of various plants (MATHUR, 1946; MURALIRANGAN, 2004).

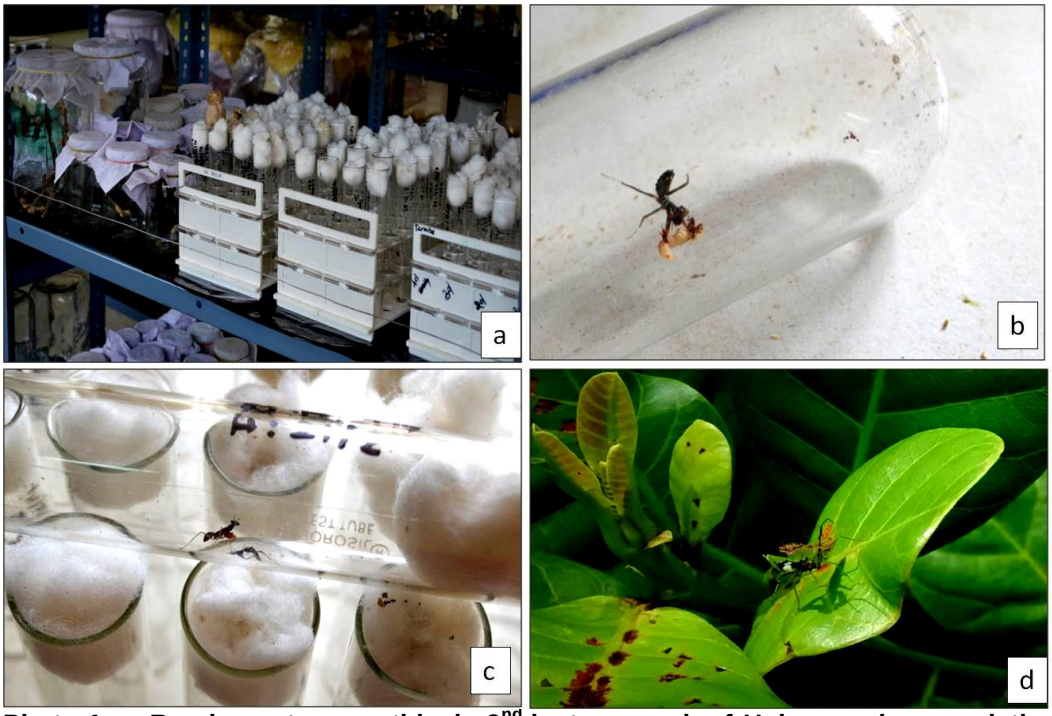

Photo-1: a. Rearing setup mantids, b. 2nd instar nymph of *H. brunneriana* predating a wax moth larva 2nd instar nymph of *E. pulchra* d. 5th instar nymph of *E. pulchra* predating a TMB in field

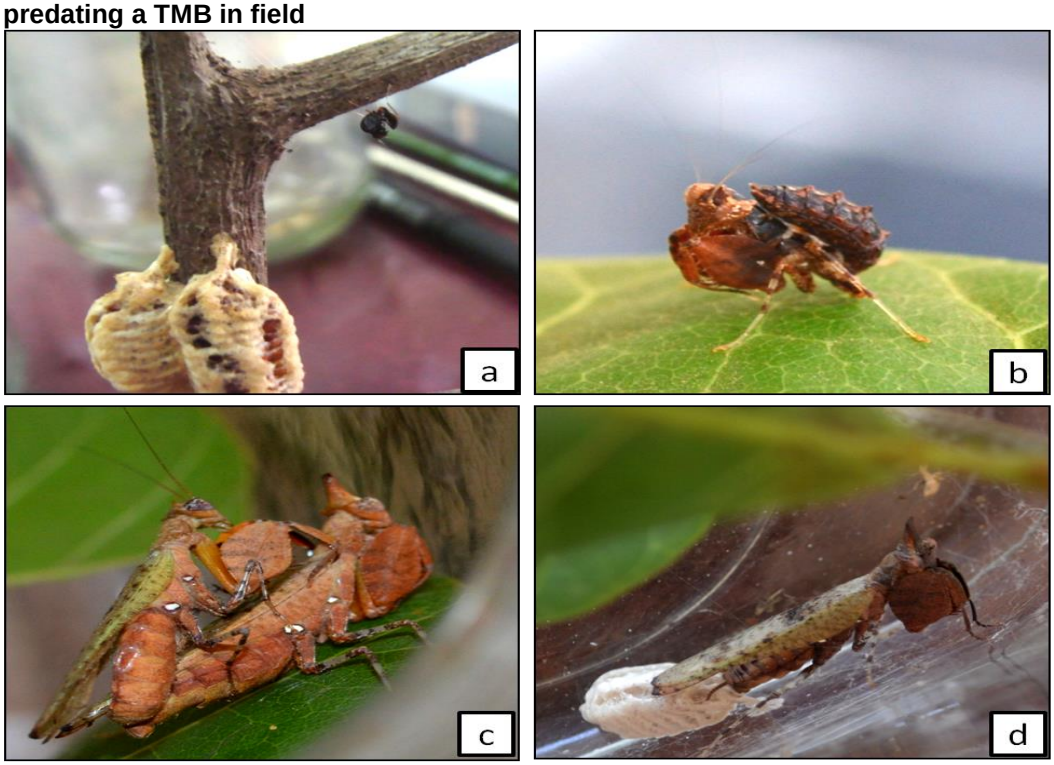

Photo-2: Life stages of *Hestiasula brunneriana* a. ootheca and the just jatched nymph, b.3rd instar nymph, c. a mating pair d. oviposition by female mantid.

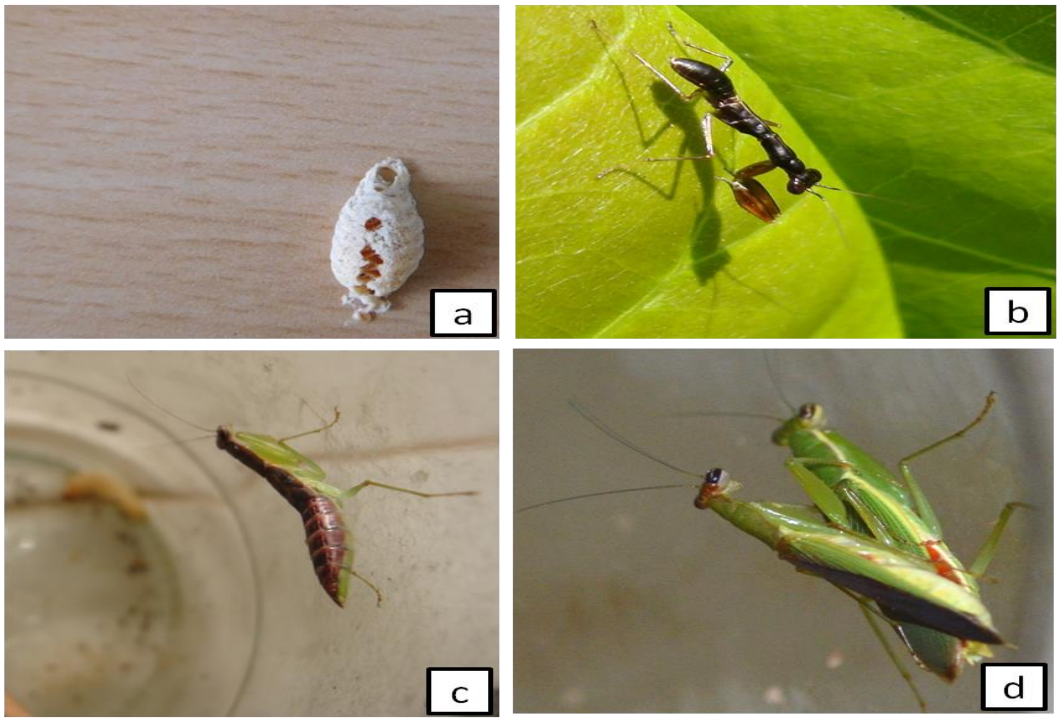

Photo-3: Life stages of *E. pulchra* a. ootheca after hatching, b. 2rd instar nymph, c. 4th instar nymph d. a mating pair

During the present study, different stages of *H. brunneriana* were noticed throughout the year, and more numbers were sighted during August to February. While, nymphs and adults of *E. pulchra* were noticed mid rainy season onwards (July to August) and frequent during flushing and flowering period of cashew. This is in support of an earlier report, which stated that most of the mantids in India reach maturity from July to November, and adults were recorded between April and December (SURESHAN *et al*. 2007). In Punjab, *Euantissa ornata* was observed during summer, *H. brunneriana*, *L. indica* and *E. guttattipennis* during August to December (MATHUR, 1946).

In general, most aspects of the praying mantids are not studied properly except few species, because of difficulty in rearing and maintenance of mantids. The rearing studies conducted on mantids generally involve more than one prey (HURD *et al*., 2004, BATISTON *et al*., 2008, MAXWELL, 2010). But, the present method involved greater wax moth larvae alone as prey which is also important in laboratory studies to overcome any influence of prey on biological parameters of insects reared (EUBANKS and DENNO, 2000, ANDERSON and CUMMINS, 1979).The present study indicates the suitability of wax moth larvae for successful rearing of these two mantids species. Hatchability percentage was around 90 in both species. A few eggs could not hatch, which might be due to developmental failure as observed in *Stagmomantis limbata* Hahn. (HARRIS and MARON, 2000). In both species, nymphal mortality was high in early instars compared to late instars. As there was no food limitation for the nymphs in lab conditions, high mortality of the early instars could be apparently due to their high vulnerability as reported by YAGER (1999). There were seven nymphal instars in *H. brunneriana* and six in *E. pulchra*. Commonly, number of instars vary with species, which was observed as six in

Orthodera novaezealandiae (Colenso) (PURKAYASTHA, 1999), seven in *Tenodera aridifolia* (Stoll) (HURD *et al*., 2004).

Nymphal developmental period was comparatively more in *H. brunneriana* with 87.66±1.01 and 90.06±0.87 days, respectively for male and female, whereas, in *E. pulchra*, it was just 58.5±0.60 and 57.08±0.98 days, respectively. Nymphal developmental period of 3 - 6 months had been recorded in *O. novaezealandiae* (PURKAYASTHA, 1999). Nymphal survival was high in *H. brunneriana* (85-95 %) compared to *E. pulchra* (10-20 %). Because unlike *E. pulchra* nymphs, the nymphs of *H. brunneriana* could effectively feed on wax moth larvae even bigger than them and survive better. Under captive breeding of *E. pictipes* using wax moth larvae as prey, nymphal survival percentage of 39 - 46 % was recorded (VANITHA *et al*., 2016). Preoviposition period was 10-15 days in *H. brunneriana* and 10-14 days in *E. pulchra,* which was 45 and 60 days in Peruvian Shield mantis, *Choeradodis rhombicollis* (Latreille) (KONIGIN, 2012) and Chinese praying mantis, *T. aridifolia* (HURD *et al*., 2004), respectively. Fecundity was 5-7 in *H. brunneriana* and 8-15 in *E. pulchra*. However, fecundity of only one was reported in *T. aridifolia* (MINER, 2013).

Pre-mating cannibalism was noticed when the male was released before the sexual maturity as observed in *Pseudomantis albofimbriata* (Stal.) (BARRY, 2004) and *E. pictipes* (VANITHA *et al*., 2016). According to ARNQVIST and HENRIKSSON (1997), pre-mating cannibalism by females may be the result of high and indiscriminate aggressiveness during the early life stages. As observed in other mantids (ROEDER, 1935), the sexual behaviour consisted of two distinct phases. Both patterns of male approach were also observed in *T. a. sinensis, P. albofimbriata* (LELITO and BROWN 2006a, BARRY *et al.,* 2009). Duration of copulation was long in *H. brunneriana* (5.30 – 5.45 h) compared to *E. pulchra* (35-50 min). Similarly, longer copulation duration of 5-6 hours had been observed in *E. pictipes* (VANITHA *et al*., 2016). Though, 90 % of the mantid species participate in sexual cannibalism (WILDER *et al*., 2009), it was not observed in *H. brunneriana* and *E. pulchra*. This could also be due to the healthy condition of females resulted by regular feeding in lab making them less cannibalistic.

In summary, the study provides insights in to biological traits of two mantid species and their successful mass rearing. High fertility, shorter life cycle, multivoltinism, and suitability for captive breeding is the advantageous characteristics of these two species for mass culture and inundative releases. These mantids appear to be potential candidates for biological control of certain insect pests of cashew. Nevertheless, strategies are required to ensure establishment of mantids in sufficient numbers cashew plantations so as to assess their pest management efficiency.

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SELECTION OF SUITABLE AND ECONOMIC SUBSTRATE FOR MASS PRODUCTION OF FUNGUS, *BEAUVERIA BASSIANA*

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ABSTRACT: The experiment on mass production studies was conducted in the Entomology Laboratory, College of Agriculture, JNKVV, Jabalpur during *Rabi* 2013-14 on fourteen substrates to determine a suitable medium for growth and sporulation. During this period the maximum and minimum temperature of the laboratory were 39.55 ± 3.65 ^{0}C and 29.6 ± 6.4 ^{0}C, respectively while morning and evening relative humidity were 51 ± 19 % and 24.5 ± 8.5 %, respectively. The observations were recorded on 10th, 20thand 30th days after inoculation. The mean spore load recorded on solid and liquid substrates were 2.20 ×10^7 spores and 1.75 ×10^7 spores, respectively. However, among the different solids substrates, broken grains recorded maximum spore load (2.61 ×10^7 spores), followed by husk (2.37 ×10^7 spores), bran (2.08 ×10^7 spores) and lowest on whole grains (1.78 ×10^7 spores), respectively. Among the different substrates evaluated significantly highest conidial count (4.77 × 10^7 spores/ml) was recorded on broken rice media having production cost of ₹ 3.44 followed by wheat husk (4.40 × 10^7 spores/ml), broken wheat (2.57 × 10^7 spores/ml) and wheat bran (2.20 × 10^7 spores/ml) having production cost of ₹ 3.16, ₹5.62 and ₹7.27, respectively.

Key words: Mass production, Economic Substrate, *Beauveria bassiana*

INTRODUCTION

Biopesticides are pesticides derived from natural materials like animals, plants, bacteria, and certain minerals including botanicals, microbials, natural enemies and semio-chemicals (SAKHRIE *et al*., 2016). Biopesticides are effective to control insects, plant diseases and weeds, as well as safe to human and environmental in addition to their important role in managing pesticide resistance, niche markets and minimizing the use of synthetic pesticides. These are usually inherently less toxic than conventional pesticides and generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects, and mammals (GUPTA and DIXSHIT, 2010). *Beauveria bassiana* is a fungus that grows naturally in soils and acts as a parasite on various arthropod species, causing white muscardine disease; it thus belongs to the entomopathogenic fungi. It is being used as a biological insecticide to control a number of pests such as termites, thrips, whiteflies, aphids and beetles. When its microscopic spores comes into contact with the body of an insect host, they germinate, penetrate the cuticle and grow inside, killing the insect within a few days (ROYCHOUDHARY and DADWAL, 2010; BARBARIN *et al*., 2012). Besides, larvae, eggs and pupae of some lepidopterous pests are susceptible to *B. bassiana* (PANDEY, 2003). We studied the suitablity of certain substrate for mass multiplication of *Beauveria bassiana* which are not adequately explored.

MATERIALS AND METHODS

The laboratory studies were conducted in the Entomology Laboratory, College of Agriculture, JNKVV, Jabalpur during *Rabi* 2013-14, under completely randomized design.

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There were 14 treatments comprising of whole grains, broken grains, bran, husk, and water soaked (Table-1). Media were prepared using whole and broken grains as well as bran and husk of wheat, *Triticum aestivum* (L); rice, *Oryza sativa* (L); maize, *Zea mays* (L); sorghum, *Sorghum vulgare* Pers. to estimate the sporulation of *Beauveria bassiana*, at 25ºC. For this purpose, 100 g of each grain was washed and soaked in water overnight except rice which was soaked for 2 – 3 hours. The excess water was drained by decanting and shade drying them for half an hour to further remove the excess moisture. The grains were packed separately in 250 mL conical flask, with cotton plug and auto calved at 15 psi for 30 minutes. After cooling, 5 mm fungal disc was inoculated into each flask under laminar air flow chamber. They were incubated in BOD incubator at 25ºC for 15 days. Two replications were maintained for each treatment. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the grain and to break the mycelial mat.

Using bran & husk, 100 g each of the bran and husk, 50 ml of sterile distilled water was added in a 250 ml conical flask. The substrates were sterilized in an autoclave at 15 psi for 30 minutes. After sterilization the substrates were artificially inoculated with 5 mm fungal disc under laminar air flow chamber. Each treatment was replicated two times. After inoculation, the flasks were incubated at 25 ^{0}C for 15 days. The conical flasks were shaken daily for the uniform growth of the fungus. Cost of production was calculated based on market price of different substrates. All the data was subjected to statistical analysis after appropriate transformation as suggested by GOMEZ and GOMEZ, 1984. The observations spore counts were recorded on 10th, 20thand 30th days after inoculation and the data presented in Table-1.

RESULTS AND DISCUSSION

a. Ten days after inoculation: Among the different substrates evaluated, significantly highest conidial count (3.8 × 10^7 spores/ml) was recorded on broken rice media and was followed by wheat husk (2.9 × 10^7 spores/ml), but they differed significantly from each other. The next group of substrates were broken wheat (1.8 × 10^7 spores/ml), followed by wheat bran (1.7 × 10^7 spores/ml), whole sorghum (1.6 × 10^7 spores/ml), but they did not differ significantly from each other. The next group of substrates were water soaked rice (1.5 × 10^7 spores/ml), followed by rice bran (1.4 × 10^7 spores/ml), both of them were at par with each other. The next group of substrates were whole rice (1.2× 10^7 spores/ml), broken sorghum (1.2× 10^7 spores/ml), whole wheat (1.1 × 10^7 spores/ml), whole maize (1.1 × 10^7 spores/ml), broken maize(1.1 × 10^7 spores/ml) and water soaked wheat (1 × 10^7 spores/ml), but they did not differ significantly from each other and least spore count was recorded in rice husk (0.2 × 10^{7}spores/ml).

b. Twenty days after inoculation: Among the different substrates evaluated, significantly highest conidial count (4.9 × 10^7 spores/ml) was recorded on broken rice media followed by wheat husk (4.8 × 10^7 spores/ml), but they did not differ significantly from each other. The next group of substrates were wheat bran (2.4 × 10^7 spores/ml), whole sorghum (2.1 × 10^7 spores/ml) and rice bran (2.0 × 10^7 spores/ml), but they did not differ significantly from each other. The next group of substrates were whole grain rice (1.9 × 10^7 spores/ml), followed by water soaked rice (1.9 × 10^7 spores/ml), whole wheat (1.7× 10^7 spores/ml), whole maize (1.7× 10^7 spores/ml), water soaked wheat (1.6 × 10^7 spores/ml), broken sorghum (1.5 × 10^7 spores/ml),broken maize (1.4 × 10^{7}spores/ml), but all were at par with each other and least spore count was recorded in rice husk (0.3 × 10^{7}spores/ml).

Table-1: Mass production of *Beauveria bassiana*on different substrates

Treatment number	Groups (Media)	Substrates	Spore count(1 ×10′spores/ml) at different days after inoculation				Rate of increase in growth of *B. bassiana* (%) (DAI)	
			10th day	20th day	30th day	Mean	10 to 20 DAI	20 to 30 DAI
T_1	Whole grains (Solid media)	Wheat, *Triticum aestivum* (L)	1.10	1.70	2.10	1.63	35.42 (36.81)	19.09 (26.24)
T_2		Rice, *Oryza sativa* (L)	1.20	1.90	2.20	1.77	36.67 (37.54)	13.64 (21.75)
T_3		Maize, *Zea mays* (L)	1.10	1.70	2.10	1.63	35.42 (36.81)	18.64 (25.34)
T_4		Sorghum, *Sorghum bicolour* (L)	1.60	2.10	2.50	2.07	23.64 (29.34)	16.03 (23.93)
T_5	Broken grains (Solid media)	Wheat	1.80	2.70	3.20	2.57	33.24 (35.48)	15.63 (23.56)
T_6		Rice	3.80	4.90	5.60	4.77	22.50 (28.63)	12.50 (21.05)
T_7		Maize	1.10	1.40	1.90	1.47	21.43 (27.57)	26.11 (30.99)
T_8		Sorghum	1.20	1.50	2.20	1.63	19.64 (26.44)	31.82 (34.56)
T_9	Bran (Solid media)	Wheat	1.70	2.40	2.50	2.20	27.86 (31.96)	4.99 (4.49)
T_{10}		Rice	1.40	2.00	2.50	1.97	29.29 (32.90)	20.19 (26.88)
T_{11}	Husks (Solid media)	Wheat	2.90	4.80	5.50	4.40	39.58 (39.26)	12.70 (21.23)
T_{12}		Rice	0.20	0.30	0.50	0.33	25.00 (24.67)	41.67 (40.42)
T_{13}	Water soaked (Liquid media)	Wheat	1.00	1.60	2.20	1.60	36.51 (37.33)	27.50 (31.93)
T_{14}		Rice	1.50	1.90	2.30	1.90	21.11 (27.69)	17.42 (24.99)
		SE m ±	0.08	0.15	0.10	0.09	6.29	6.08
		CD at 5%	0.26	0.47	0.29	0.28	NS	NS

Max. temp.= 39.55 ± 3.65 °C, Min. temp.= 29.6 ± 6.4 °C, Morning RH(%) =51 ± 19, Evening RH(%) = 24.5 ± 8.5
() = Figures in the parentheses are arcsin transformed values NS = Non significant DAI-Days after inoculation

Table-2: Economics of mass production of *Beauveria bassiana* on/in different substrates

Treatment code	**Media**	**Group**	**Substrates**	**Mean spore count (1×10^7spore/ml)**	**Cost of substrate /100g (Rs)**	**Cost of production of *B. bassiana* 1×10^7spores/ml (Rs)**
T_1	**Solid Media**	Whole grains	Wheat, *Triticum aestivum*	1.63	2.00	8.87
T_2			Rice, *Oryza sativa*	1.77	4.00	9.30
T_3			Maize, *Zea mays*	1.63	3.00	9.43
T_4			Sorghum, *Sorghum bicolour*	2.07	2.5	7.23
T_5		Broken grains	Wheat	2.57	2.00	5.62
T_6			Rice	4.77	4.00	3.44
T_7			Maize	1.47	3.00	10.54
T_8			Sorghum	1.63	2.50	9.11
T_9		Bran	Wheat	2.20	3.60	7.27
T_{10}			Rice	1.97	0.50	6.57
T_{11}		Husks	Wheat	4.40	1.50	3.16
T_{12}			Rice	0.33	0.60	39.39
T_{13}	**Liquid Media**	Water soaked	Wheat	1.60	2.00	9.06
T_{14}			Rice	1.90	5.00	9.19
			SEm ±	0.09	--	0.33
			CD at 5%	0.28	--	1.03

c. Thirty days after inoculation: Among the different substrates evaluated, significantly highest conidial count (5.6×10^7 spores/ml) was recorded on broken rice media, followed by wheat husk (5.5×10^7 spores/ml), but both of them were at par with each other. The next group of substrates included whole sorghum (2.5×10^7 spores/ml), wheat bran (2.5×10^7 spores/ml), rice bran (2.5×10^7 spores/ml), water soaked rice (2.3×10^7 spores/ml), but all were at par with each other. The next group of substrates were whole rice (2.2×10^7 spores/ml), broken sorghum (2.2×10^7 spores/ml), water soaked wheat (2.2×10^7 spores/ml), whole wheat (2.1×10^7 spores/ml), whole maize (2.1×10^7 spores/ml), broken maize (1.9×10^7 spores/ml), but they did not differ significantly from each other. The least spore count was recorded on rice husk (0.5×10^7spores/ml).

Among the different substrates evaluated significantly highest conidial count (4.77×10^7 spores/ml) was recorded on broken rice media followed by wheat husk (4.40×10^7 spores/ml), broken wheat (2.57×10^7 spores/ml) and wheat bran (2.20×10^7 spores/ml) and they differed significantly from each other. The next group of substrates included whole sorghum (2.07×10^7 spores/ml), rice bran (1.97×10^7 spores/ml) and water soaked rice (1.90×10^7 spores/ml), but all the three substrates were at par with each other. The next group of substrates included whole rice (1.77×10^7 spores/ml), whole wheat (1.63×10^7 spores/ml), whole maize (1.63×10^7 spores/ml), broken sorghum (1.63×10^7 spores/ml) and water soaked wheat (1.60×10^7 spores/ml), but they did not differ significantly from each other. The least spore count was recorded on rice husk (0.33×10^7spores/ml).

Further, rate of increase in growth of *B. bassiana* calculated (Table-1) revealed that from 10^{th} to 20^{th} days after inoculation among different substrates was found to be non significant. The highest rate of increase in growth of fungus was recorded on wheat husk (39.58%) followed by whole rice (36.67%), water soaked wheat (36.51%), whole wheat (35.42%), whole maize (35.42%), broken wheat (33.24%), rice bran (29.29%), wheat bran (27.86%), rice husk (25.00%), whole grain sorghum (23.64) broken rice (22.50%), broken maize (21.43%) and water soaked rice (21.11%) and least growth rate of the fungus was recorded on broken sorghum (19.64%). The rate of increase in growth of *B. bassiana* from 20^{th} to 30^{th} days after inoculation among different substrates was found to be non significant. The highest rate of increase in growth was recorded on rice husk (41.67%) followed by broken sorghum (31.82%), water soaked wheat (27.50%), broken maize (26.11%), rice bran (20.19%), whole wheat (19.09%), whole maize (18.64%), water soaked rice (17.42%), whole grain sorghum (16.03%), broken wheat grain (15.63%), whole grain of rice (13.64%), wheat husk (12.70%), broken rice (12.50%) and least growth rate was recorded on wheat bran (4.99%).

Growth of *B. bassiana* on different group of substrates (Table-1) indicated that at 10 DAI, among the solid and liquid substrates, the former recorded maximum spore load of (1.59×10^7 spores) while in the later it was 1.25×10^7 spores. However, among the different solid substrates, broken grains recorded maximum spore load (1.98×10^7 spores) followed by bran and husk (1.55×10^7 spores each) and lowest on whole grains (1.25×10^7spores), respectively. At 20 DAI, among the solid and liquid substrates, the mean spore load was 2.28×10^7 spores and 1.75×10^7 spores, respectively. However among the different solid substrates, broken grains recorded maximum spore load (2.63×10^7 spores), followed by husks (2.55×10^7 spores), bran (2.20×10^7 spores) and lowest on whole grains (1.85×10^7 spores), respectively. Whereas at 30 DAI the mean spore load recorded on solid and liquid substrates were 2.73×10^7 spores and 2.25×10^7 spores, respectively. However, among the different solid substrates, broken grains recorded

maximum spore load (3.23×10^7 spores) followed by husks (3.00×10^7 spores), bran (2.50×10^7 spores) and lowest on whole grains (2.23×10^7 spores), respectively.

The mean spore load recorded on solid and liquid substrates were 2.20×10^7 spores and 1.75×10^7 spores, respectively. However, among the different solids substrates, broken grains recorded maximum spore load (2.61×10^7 spores), followed by husk (2.37×10^7 spores), bran (2.08×10^7 spores) and lowest on whole grains (1.78×10^7 spores), respectively. The present findings are in agreement with the findings of (IBRAHIM and LOW (1993), MAZUMDER *et al.* (1995), PUZARI *et al.* (1998), SHARMA *et al.* (2002), MONDAL and BHATTACHARYA (2004), RAO *et al.* (2005), SAHAYARAJ and NAMASIVAYAM (2008), KALIDAS (2010), PANDEY and KANAUJIA (2010), RAJANIKANTH *et al.* (2010 and 2011), SACHIN KUMAR *et al*. (2011), SUASA-ARD *et al.* (2011), RISHI *et al.* (2013) and YADAV *et al.* (2013). They also reported that rice was found to be the best solid substrate for spore production and their viability was also high but they further emphasized that fungus also grows equally well on maize or other grains followed by bran and husk also.

Cost of production of 1×10^7spores was calculated for all treatments and the data are presented in Table-2. The cost of production on different substrates significantly varied from each other. Significantly lowest production cost was recorded on wheat husk media (₹ 3.16), this was followed by broken rice (₹ 3.44), but they were at par with each other. The next substrate was rice bran (₹ 6.57) followed by whole sorghum (₹ 7.23) and wheat bran (₹ 7.27), but all the three were at par with each other. The next substrate was whole wheat grain (₹ 8.87), followed by water soaked wheat (₹ 9.06), broken sorghum grain (₹ 9.11), water soaked rice (₹ 9.19), whole rice grain (₹ 9.30), whole maize grain (₹ 9.43), but they did not differ significantly from each other. The highest production cost was recorded in rice husk (₹ 39.39).

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BIO-EFFICACY OF BUPROFEZIN 25%SC AGAINST SUCKING PESTS IN PADDY ECOSYSTEM IN KARNATAKA

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ABSTRACT: Sucking insects are one of the major factors for reduction of production and productivity of rice in India. Chemical method is one of the reliable and sole method of management of the insect pests. In the present investigation, Buprofezin was evaluated against sucking pests and natural enemies in rice ecosystem. Buprofezin 25SC @ 225 g a.i/ha recorded lowest BPH population of 8.06 BPH/hill and percent reduction of BPH population was 74.19 % 15 days after second spray. Similarly the treatment Buprofezin 25SC @ 225 g a.i/ha recorded lowest WBPH population with 7.21 WBPH/hill 15 days after second spray and percent reduction of WBPH over control was 81.47%, which was statistically on par with Buprofezin 25SC @200 g a.i./ha(9.61 WBPH /hill and per cent reduction was 75.30%). Whereas green leaf hopper population was lowest with 2.80 GLH/hill in the treatment Buprofezin25SC @225g a.i/ha and percent reduction over control was 83.35% at 15 days after second spray. The highest paddy yield was recorded in Buprofezin 25SC@ 225 &200 g a.i/ha with 54.93 and 52.11qt/ha respectively. The effect of Buprofezin on predators like mirid bugs and spiders was not significantly different compare to other chemicals.

Key words: Paddy, Plant hopper, White backed plant hopper, bioefficacy, Buprofezin

INTRODUCTION

Rice (*Oryza sativa* L.) is grown mainly in Asian countries like China, India, Japan, Korea Republic, Srilanka, Pakistan, Bangladesh, etc. More than 90 % of rice is produced and consumed in Asian countries. India ranks first in the world in rice area with 44.3 million hectares followed by China with 29.3 million ha. However, with respect of productivity, India occupies 15th with 3.01 t/ ha of rough rice. The major reason for low productivity in India is the losses due to insect pests and diseases. The overall losses due to insect damage in rice have been estimated to be 25 % (DHALIWAL *et al*., 2010). More than 100 species of insects have been reported to ravage the rice crop, of these 15-20 are considered to be economically important (HEONG AND HARDY, 2009). Among sucking insect pests, brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) and whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae) are the most economically important on rice crop (SINGH *et al*. 2002). Among the biotic stresses, the brown planthopper (BPH) *Nilaparvata lugens* (Stal.) is one of the most destructive pests of rice in Asia (PARK *et al.*, 2008). The brown planthopper causes direct damage to rice plant by sucking plant sap and indirect damage by acting as a vector for viral diseases. The brown planthopper suck sap by plant stem leads to reduction in plant height, plant dry weight, severe injury usually resulted in wilting of the plants which is called hopper burn (CHEN AND CHENG, 1978) and found at all stages of plant development (DALE, 1994), hence crop loss is usually considerable and complete destruction of crop occurs in severe cases (PRAKASH *et al*., 2014).

To control rice pest farmers solely depends on chemical method. However, indiscriminate use of insecticides and their dosages has led to many problems like elimination of natural predators, environmental pollution (BALAKRISHNA and SATYANARAYANA, 2013), resistance and resurgence (KRISHNAIAH *et al*., 2006). Hence there is need to use eco- friendly and effectual chemicals with novel mode of action, which can fit very well in IPM programme. A new approach towards this step is use of insect growth regulators (IGR's) for the management of insect pests. Among these, buprofezin, a chitin synthesis inhibitor, which acts specifically on sucking pests, has been used effectively against various sucking insect pests such as whitefly on cotton planthopper on rice, jassid on brinjal and mealybug on grapes and cotton. However, its effectiveness against BPH and WBPH on rice crop is still lacking. Therefore, we studied the bioefficacy of Bufrofenzin against plant hopers in rice ecosystem.

MATERIALS AND METHODS

Evaluations of the Buprofezin 25%SC against paddy hoppers were undertaken in an experimental block, Agricultural Research Station, Kawadimatti, Surpur, Yadgiri, Karnataka during Rabi 2011-12. The experiment was laid out in a randomized block design (RBD) with four replications. The test chemical, Buprofezin 25%SC was tested at three different concentration *viz*., 175,200 and 225 g a.i/ha for bio-efficacy. The test chemical, Buprofezin25SC was compared with standard checks *viz.,* Phorate 10%G@1500 g a i/ha against paddy hoppers and an untreated control. Treatments were imposed two times based on pest population build-up (above ETL). All the agronomic packages were followed as per recommended package of practices of UAS Raichur. Observations recorded from ten tagged plants per plot. Observations on paddy hoppers BPH (Brown Plant Hopper), WBPH (White Backed Plant Hopper) and GLH (Green Leaf Hopper) and natural enemies were recorded on whole plant basis from ten tagged plants / per plot. Observations for all insects were recorded at 5, 10 and 15 days after each application. Yield was recorded per plot basis at harvest. The data collected from two sprays was averaged and expressed on per plant basis. The yield data collected from the each plot was extrapolated on hectare basis. The treatments were subjected to statistical analysis by single factor ANOVA and were compared by following Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The bio-efficacy of Buprofezin at different concentrations caused significant reduction in brown plant hoppers, white backed plant hoppers and green leaf hoppers.

BPH: The treatment Buprofezin 25SC @ 225 g a.i/ha recorded brown plant hopper 8.61 BPH /hill at 15 days after first spray and percent reduction of BPH over control was 77.21%, which was statistically on par with Buprofezin 25SC @200 g a.i./ha (9.21 BPH /hill and percent reduction was 75.51%). The next best treatment was

Table-1: Bio efficacy of Buprofezin25%SC against paddy brown plant hopper

Treatments	Dosage	5DAS				10DAS				15DAS			
		1st Spray		2nd Spray		1st Spray		2nd Spray		1st Spray		2nd Spray	
		No of BPH	% Redn.	No of BPH	% Redn.	No of BPH	% Redn.	No of BPH	% Redn.	No of BPH	% Redn.	No of BPH	% Redn.
Buprofezin 25%SC	175	21.52 (4.74)	41.10b (39.87)	18.99c (4.46)	51.46bc (45.83)	14.92 (3.98)	52.19c (46.25)	16.37 (4.17)	59.30bc (50.40)	12.54 (3.67)	66.66b (54.87)	12.93 (3.72)	58.61b (49.99)
Buprofezin 25%SC	200	18.26 (4.38)	50.02a (45.01)	16.31 (4.15)	58.31b (49.86)	11.71 (3.56)	62.47b (52.22)	13.54 (3.79)	66.32b (54.56)	9.21 (3.19)	75.51a (61.35)	9.13 (3.17)	70.77a (57.43)
Buprofezin 25%SC	225	16.10 (4.13)	55.93a (48.41)	12.33 (3.64)	68.48a (55.84)	9.30 (3.20)	70.20a (56.95)	9.33 (3.21)	76.80a (61.30)	8.61 (3.10)	77.21a (61.48)	8.06 (3.00)	74.19a (59.46)
Phorate 10%CG	1500	23.21 (4.92)	36.48b (37.12)	24.46 (5.00)	37.49d (39.19)	22.23 (4.81)	28.77e (32.32)	19.13 (4.48)	52.44c (46.40)	22.57 (4.85)	40.00d (39.16)	16.23 (4.15)	48.04c (43.87)
Thiamethoxam 25%WG	25	18.10 (4.36)	50.46a (45.26)	18.46 (4.41)	52.82b (46.61)	14.36 (3.91)	53.98c (47.28)	14.42 (3.90)	64.15b (53.34)	12.20 (3.63)	67.57b (55.39)	11.86 (3.58)	62.03b (52.03)
Monocrotophos 36SL	360	21.62 (4.75)	40.83b (39.58)	22.22 (4.81)	43.46cd (41.22)	19.30 (4.50)	38.16d (38.01)	20.10 (4.59)	50.01c (45.00)	17.52 (4.30)	53.42c (46.96)	17.44 (4.29)	44.17c (41.61)
T7. Untreated check		36.54 (6.11)		39.13 (6.63)		31.21 (5.68)		40.23 (6.41)		37.62 (6.19)		31.24 (5.65)	
SEM ±		0.14	1.61	0.18	1.68	0.19	1.44	0.14	2.07	0.12	1.94	0.13	1.72
CD at 5%		0.41	4.83	0.53	5.06	0.57	4.32	0.43	6.21	0.37	5.83	0.39	5.17

Thiamethoxam 25WG 25 @ g a.i/ha with 12.20 BPH /hill and 67.57% reduction of BPH over control, which was on par with Buprofezin 25SC @175 g a.i/ha(12.54 BPH /hill and percent reduction over control was 66.66). However the highest population of hoppers (37.62 BPH/hill) was recorded in untreated control. In second spray also, same trend was observed (Table-1). Thus Buprofezin 25SC @200g a.i/ha was found significantly superior to other treatments against BPH.

WBPH: Buprofezin 25 SC at different concentrations caused significant reduction in WBPH population, compared to untreated control plots (Table-2). Fifteen days after second application, lowest WBPH Population was recorded in Buprofezin 25SC @ 225g a.i/ha (7.21WBPH/hill, 81.47 percent reduction over control) which was on par with Buprofezin 25SC 200g a.i/ha (9.61WBPH /hill and 75.30 percent reduction over control). Both the treatments were significantly superior to all other treatments. The next best treatment was Thiamethoxam 25WG 25 g. a.i/ha recorded 9.81WBPH/hill and 74.75 percent reduction over control. However the highest population was recorded in untreated control (38.92WBPH/hill). ThUs Buprofezin 25SC @200 and 225 g. a.i/ha was significantly superior to other treatments against WBPH.

GLH: The results revealed that Buprofezin 25SC at different concentrations caused significant reduction of green leaf hopper over control. The treatments with Buprofezin 25SC @225g a.i/ha recorded lowest GLH population 2.80 GLH/hill and per cent reduction over control was 83.35% at 15 days after second spray, which was on par with Thiamethoxam 25WG 25 g a.i/ha (3.50GLH/hill and 79.19% reduction over control). Next best treatment was Buprofezin25SC @200g a.i/ha (6.21GLH/hill and 63.07% reduction over control). However untreated control was recorded highest green leaf hopper population (16.82GLH/hill) (Table-3). Thus Buprofezin 25SC @225g a.i/ha was also found significantly superior to other treatments against GLH.

Buprofezin is the first insect growth regulator (IGR) registered in the world for the control of rice pests. It shows high activity on homopterous pest insects such as rice planthoppers and leafhoppers without any adverse effects on their predators and parasitoids. Since the chemical inhibits larval molting, egg-laying and/or induces oviposition of unhatchable eggs, it suppresses the population density of hoppers even in the progeny of the treated generation with long lasting activity in the paddy field. Buprofezin causes no resurgence of hoppers by itself or with the combination of other insecticides as reported by KONNO (1990).

Yield: The highest paddy yield was recorded in Buprofezin 25SC @ 225 and 200 g a.i/ha (54.93 and 52.11qt/ha respectively) and were significantly superior over other treatments. The yield in other treatments varied from 41.04 to 50.11q/ha, while untreated control recorded the lowest yield of 29.67q/ha (Table-4). The results of the present investigation are in line with the reports of SHERA and SARAO (2016), who tested field efficacy of Buprofezin 25 SC @ 625, 750 and 875 ml/ha against brown planthopper (BPH) and white backed plant hopper (WBPH). The reduction in BPH population after 7 and 10 days of spray was 81.23 and 86.55 % in Buprofezin @ 825 ml/ha as against 77.85 and 85.18 % in its lower dose (750 ml/ha). Buprofezin @ 750 and 825 ml/ha resulted in 79.02 and 82.96 % reduction in WBPH at 7 DAS and 86.51 and 88.18 % at 10 DAS, respectively.

Table-2: Bio efficacy of Buprofezin25%SC against paddy WBPH (white backed brown plant hopper).

Treatments	Dosage	5DAS				10DAS				15DAS			
		1st Spray		2nd Spray		1st Spray		2nd Spray		1st Spray		2nd Spray	
		No of BPH	% Redn.	No of BPH	% Redn.	No of BPH	% Redn.	No of BPH	% Redn.	No of BPH	% Redn.	No of BPH	% Redn.
Buprofezin 25%SC	175	26.72 (5.26)	49.67cd (44.80)	19.45 (4.50)	65.97bc (54.34)	15.25 (4.03)	57.90bc (49.54)	16.51 (4.17)	60.33bc (51.25)	13.26 (3.77)	(65.32)c (53.92)	13.21 (3.76)	66.05bc (54.40)
Buprofezin 25%SC	200	21.21 (4.71)	60.05ab (50.86)	13.66 (3.81)	76.10a (60.82)	10.05 (3.32)	78.41a (62.88)	12.33 (3.65)	70.37ab (57.18)	11.61 (3.55)	76.33ab (60.97)	9.61 (3.25)	75.30a (60.44)
Buprofezin 25%SC	225	20.36 (4.60)	61.65a (51.73)	11.32 (3.52)	80.19a (63.94)	8.71 (3.04)	81.29a (64.79)	9.30 (3.20)	77.65a (61.78)	9.20 (3.19)	81.24a (64.33)	7.21 (2.86)	81.47a (64.50)
Phorate 10%CG	1500	34.26 (5.96)	35.48e (36.53)	20.33 (4.61)	64.43c (53.38)	28.01 (5.38)	39.84d (39.13)	17.50 (4.29)	57.95c (49.59)	31.30 (5.68)	36.18e (36.89)	14.60 (3.94)	62.48c (52.30)
Thiamethoxam25%WG	25	24.32 (5.02)	54.19bc (47.12)	14.21 (3.87)	75.13ab (60.08)	19.60 (4.53)	67.24b (55.08)	11.80 (3.57)	71.64a (57.82)	17.01 (4.24)	72.96b (58.86)	9.81 (3.28)	74.75ab (59.86)
Monocroto-phos 36SL	360	29.55 (5.52)	44.35d (41.75)	22.87 (4.88)	59.98c (50.52)	23.21 (4.92)	50.15c (45.08)	17.66 (4.31)	57.56c (49.39)	22.75 (4.87)	53.61d (47.07)	15.20 (4.01)	60.09c (50.97)
Untreated check		53.10a (7.35)		57.16 (7.59)		46.56 (6.89)		41.62 (6.52)		49.05 (7.05)		38.92 (6.29)	
SEM ±		0.17	1.40	0.19	1.94	0.18	1.94	0.14	2.17	0.14	1.61	0.16	1.87
CD at 5%		0.49	4.20	0.56	5.82	0.54	5.82	0.41	6.52	0.42	4.85	0.49	5.62

Table-3: Bio-efficacy of Buprofezin25%SC against paddy GLH (green leaf hopper)

Treatments	Dosage	5DAS				10DAS				15DAS			
		1st Spray		2nd Spray		1st Spray		1st Spray		2nd Spray			1st Spray
		No of BPH	% Redn.	No of BPH	No of BPH	% Redn.	No of BPH	No of BPH	% Redn.	No of BPH	No of BPH	% Redn.	No of BPH
Buprofezin 25%SC	175	12.26bc (3.56)	30.02c (33.22)	12.27b (3.64)	30.12d (33.13)	5.72bc (2.50)	66.85bc (54.88)	9.61c (3.25)	40.89d (39.13)	3.81b (2.19)	75.13bc (61.18)	8.20bc (3.03)	51.24c (45.71)
Buprofezin 25%SC	200	10.31ab (3.36)	41.15b (39.90)	8.35ab (3.05)	52.44b (46.10)	4.52ab (2.34)	73.81b (59.21)	7.20b (2.86)	55.71c (48.28)	2.62a (1.90)	82.89ab (66.07)	6.21b (2.68)	63.07b (52.60)
Buprofezin 25%SC	225	8.13a (3.02)	53.59a (47.08)	6.82a (2.79)	61.16a (51.44)	3.10a (2.02)	82.20a (65.51)	3.42a (2.10)	78.96a (62.69)	1.80a (1.67)	88.25a (70.72)	2.80a (1.94)	83.35a (65.96)
Phorate 10%CG	1500	13.56b (3.81)	22.60d (28.38)	12.17b (3.62)	30.69cd (33.60)	7.08c (2.84)	58.98c (50.17)	12.16d (3.62)	25.21e (30.84)	7.16d (2.85)	53.26e (46.86)	11.58c (3.54)	31.15d (33.92)
Thiamethoxam 25%WG	25	11.92ab (3.35)	31.96c (34.29)	7.31a (2.88)	58.37ab (49.85)	5.20b (2.48)	69.87b (56.86)	4.70cd (2.38)	71.09b (57.64)	4.70bc (2.38)	69.32cd (56.48)	3.50a (2.12)	79.19a (63.69)
Monocrotophos 36SL	360	13.50b (3.80)	22.94d (28.61)	11.16b (3.48)	36.44c (37.10)	9.67d (3.26	43.97d (41.53)	9.02bc (3.16)	44.52d (41.78)	5.01c (2.45)	67.29d (55.11)	9.23c (3.19)	45.12c (42.19)
Untreated check		17.52c (4.30)		17.56c (4.28)		17.26c (4.27)		16.26e (4.13)		15.32e (4.02)		16.82d (4.18)	
SEM ±		0.13	1.35	0.13	1.26	0.11	1.70	0.11	1.67	0.08	1.85	0.14	
CD at 5%		0.39	4.05	0.38	3.78	0.34	5.10	0.31	5.02	0.24	5.57	0.43	5.91

Table 4: Rice Yield (Q/ha)

Treatments	Dosage	Yield(Q/ha)
T1.Buprofezin25%SC	175	46.01c
T2.Buprofezin25%SC	200	52.11ab
T3.Buprofezin25%SC	225	54.93a
T4.Phorate 10%CG	1500	41.04d
T5.Thiamethoxam25%WG	25	50.51b
T6.Monocrotophos 36SL	360	45.38cd
T7.Untreated check	---	29.67e
SEM±		1.45
CD at 5%		4.36

The yield increase over control was 15.27 and 16.50 % in buprofezin @ 750 and 825 ml/ha, respectively. The results of the present investigation are in line with the reports of SHERA and SARAO (2016), who tested field efficacy of Buprofezin 25 SC @ 625, 750 and 875 ml/ha against brown planthopper (BPH) and white backed plant hopper (WBPH). The reduction in BPH population after 7 and 10 days of spray was 81.23 and 86.55 % in Buprofezin @ 825 ml/ha as against 77.85 and 85.18 % in its lower dose (750 ml/ha). Buprofezin @ 750 and 825 ml/ha resulted in 79.02 and 82.96 % reduction in WBPH at 7 DAS and 86.51 and 88.18 % at 10 DAS, respectively. The yield increase over control was 15.27 and 16.50 % in buprofezin @ 750 and 825 ml/ha, respectively.

Similarly HEGDE and NIDAGUNDI (2009) evaluated efficacy of Buprofezin 25 SC against plant hoppers (Brown plant hopper and white-backed plant hopper) and their mirid predator, *Cyrtorhinus lividipennis*. The results clearly indicated that Buprofezin 25 SC @ 1 ml/l recorded lowest BPH population at 10 days after spray. Buprofezin at all dosages recorded significantly higher predatory mirid bug population over other treatments. Buprofezin 25 SC @ 1 ml /l recorded highest yield and was on par with Buprofezin 25 SC @ 0.75 ml/l. However, KRISHNAIAH, (1996) recorded maximum toxicity of Buprofezin on nymphs of the BPH and WBPH but moderate toxicity to GLH in the greenhouse. Combinations of Cypermethrin + Buprofezin and Deltamethrin + Buprofezin were highly effective against all three test insects. Buprofezin was safe to nymphs and adults of the predator, *Cyrtorhinus lividipennis*, (MB) but the synthetic pyrethroids and combination treatments were relatively more toxic to *C. lividipennis*. In field studies,

Cypermethrin (50 g a.i./ha) and Deltamethrin (25 g a.i./ha) caused BPH resurgence but, when used in combination with Buprofezin (100 g a.i/ha) resulted in good control of BPH. In view of relatively low mammalian toxicity, combinations of Cypermethrin + Buprofezin and Deltamethrin+ Buprofezin can be recommended for use against BPH, GLH and the leaffolder in IPM programmes in rice. The efficacy of Bufrofenzin was evaluated against BPH in rice ecosystem, Bufrofenzin 25 SC @ 200 g a.i./ha was found to be more effective in reducing BPH, followed by thiamethoxam 25 WG @ 25 g a.i./ha and Imidacloprid 200 SL @ 25 ga.i./ha reported by PRASHANT *et al*. (2015). Similar results were noticed by CHAUDHARY *et al*. (2015), who reported the treatment Buprofezin 15%+Acephate 35% WP was most effective against the sucking pests, BPH and Gundi bugs at 1500 mL/ha, the mixture significantly suppressed the population of BPH to 3.89/5 hills, respectively and gundhi bug to 1.66/five sweeps. Significantly highest yield of rice (5545 kg/h) was recorded in a combination.

Natural enemies' population: The predators like spiders and mired bugs were observed in paddy ecosystem during cropping season. One day before spray spider and mired bug population were found non significant in all treatments it indicates that predator population was uniformly distributed in all the treatments. However 10 DAS, significantly lowest predators were noticed in all the chemical treated plots (ranged from0.72 to 4.21spiders and2.12 to 6.89 mirids/hill) compared to untreated control (6.89 spiders and 15.33 mirids/hill) (Table-5).

Table-5: Bio-efficacy of Buprofezin25%SC against natural enemies' in paddy ecosystem

Treatments	**Dosage**	**Spiders/hill**		**Mirid bugs**	
		1DBS	7DAS	1DBS	7DAS
T1.Buprofezin 25% SC	175	5.23	1.98c (1.40)	11.52	4.76c (2.18)
T2.Buprofezin 25% SC	200	4.36	1.52cd (1.23)	10.33	4.21cd (2.05)
T3.Buprofezin 25% SC	225	6.21	1.21cd (1.10)	9.85	3.84cd (1.95)
T4.Phorate 10 % CG	1500	5.77	4.21b (2.05)	11.66	6.89b (2.62)
T5.Thiamethoxam 25% WG	25	5.03	1.10cd (1.04)	10.54	2.89de (1.70)
T6.Monocrotophos 36SL	360	6.81	0.72d (0.84)	9.21	2.12e(1.45)
T7.Untreated check		5.11	6.89a (2.58)	10.55	15.33a (3.87)
Sem ±			0.13		0.14
CD at 5%		NS	0.39	NS	0.43

TANAKA *et al*. (2000) evaluated toxicity of nine insecticides to predators of rice plant hoppers i.e. four spider species, mirid bug *Cyrtorhinus lividipennis* and the dryinid wasp. Insecticide susceptibilities of the spiders varied among species. Many insecticides, particularly phenthoate, imidacloprid and deltamethrin, were toxic to *C. lividipennis*. All insecticides were also tested on spiders and *C. lividipennis* in paddy fields. Deltamethrin had a destructive effect on the spider populations and may have induced a resurgence of the *Nilaparvata lugens* population. Phenthoate reduced the abundance of lycosid spiders, and ethofenprox reduced the abundance of *Tetragnatha*. The *C. lividipennis* abundance decreased to a low level in all insecticide-treated plots except those treated by buprofezin. The study concludes that Buprofezin 25SC 200 and 225g a.i/ha provided significantly superior and effective control, compared with other treatments for the management of hoppers in rice crop. Therefore Buprofezin 25SC @200 and 225g a.i/ha may be recommended for control of BPH, WBPH & GLH in rice crop. Reduction of hopper incidence provided opportunity for higher yields. No phytotoxic symptoms were observed in any of the doses tested in rice crop.

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THE INCIDENCE OF *PLUTELLA XYLOSTELLA* LINNAEUS IN CABBAGE –CROP- ECOSYSTEM OF MANIPUR VALLEY

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ABSTRACT: Replicated field trials were carried out at the College of Agriculture, CAU, Imphal during *Rabi*, 2009–2010 to study the comparative bio-efficacy of nine aqueous indigenous plant extracts and Nimbecidine against Diamond-back moth (DBM), *Plutella xylostella* Linnaeus in cabbage variety "Pride of India". The results revealed that Nimbecidine @ 2000 ml ha^{-1} proved to be the most effective with three sprays mean larval population of 9.32 /plant as against 12.85 to 22.29 /plant in the cow-urine plant extracts and 39.20/ plant in untreated check. *Melia azedarach* and *Jatropha gossypifolia* extracts each @ 12,500ml ha^{-1} with their corresponding mean larval population of 12.85 and 16.42 /plant, respectively but dffered significantly from that of Nimbecidine. The extracts of *Acorus calamus* and *Melothria perpusilla* each applied @ 12500ml ha^{-1} having 22.29 and 21.04 larvae/plant, respectively also did not afford satisfactory control of the pest. However, all the insecticidal treatments were significantly superior to untreated check in minimizing the pest population. The highest mean yield (23.12 t ha^{-1}) was obtained from Nimbecidine treated plots followed by the plots treated with *M. azedarach* (21.98 t ha^{-1}), *Artemisia nilagirica* (21.25 t ha^{1}), *Andropogon nardus* (20.22 t ha^{-1}), *J. gossypifolia* (19.85 t ha^{-1}) and *Ageratum conyzoides* (19.45 t ha^{-1}) as against 17.39 to 18.30 t ha^{-1} in rest of the five insecticidal treatmented plots and 14.35 t ha^{-1} in untreated control.. The avoidable loss due to DBM infestation varied from nil in Nimbecidine sprayed @ 2000 ml ha-1 to 37.93 per cent in untreated check. Among the plots treated with insecticides maximum avoidable loss (24.78 per cent) was computed in the plots treated with *A. calamus* extract @ 12500 ml ha^{-1}

Key words: Cabbage, *Plutella xylostella* Linnaeus, Aqueous indigenous plant extract

INTRODUCTION

Cabbage (*Brassicae oleracea* var.*capitata* Linnaeus) is one of the most important and extensively cultivated vegetable crops because of its nutritional and economical values. In Manipur, the total area under cabbage is only 1,000 hectares with a production of 10,100 metric tonnes. The productivity is only 10,100 kg/ha in the State as compared to the national productivity of 22,890 kg/ha (ANONYMOUS, 2002). Amongst several abiotic and biotic factors responsible for low productivity of cabbage, the damage caused by insect pests which infest the crop from seedling to harvest is considered as one of the major production constraints. In India, about 37 insect pests have been reported to feed on cabbage, of which the diamond-back moth (*Plutella xylostella* Linnaeus), cabbage butterfly (*Pieris brassicae* Linnaeus), cabbage aphid (*Brevicoryne brassicae* Linnaeus) and the mustard aphid (*Lipaphis erysimi* Kaltenbach) are the major limiting factors for profitable cultivation of the crop (SACHAN and GANGWAR, 1980 and LAL *et al*., 2002). Twelve species of insect pests have been observed to inflict damage on cole crops right from seedling to harvesting stage of crop growth *viz*., cut-worm (*Agrotis ipsilon* Hafner and *Spodoptera litur*a Fabricius), pea leaf miner (*Phytomyza atricornis* Meigan), aphids (*L. erysimi* , *B. brassicae* and *Myzus persicae* Sulzer), *P. xylostella* , cabbage butterflies (*P. brassicae* , *P. canidia* and *P.*

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rapae), Bihar hairy caterpillar (*Spilarctia obliqua* Walker), flea beetle (*Phyllotreta cruciferae* Goeze), pentatomid bug, (*Bagrada cruciferarum* Kirk.) and cabbage semilooper (*Plusia orichalcea* Fabricius), which are major importance in Manipur (RAM *et al*., 1981). But, amongst these insect species, *P. xylostella brassicae* is the most important and regular pests of this crop in the State. Several broad-spectrum synthetic organic insecticides are usually recommended for the effective control of these pests (SONKAR and DESAI, 1998). However, these compounds are known to evoke multifarious problems including environmental pollution, health hazards, destruction of beneficial fauna like parasitic, predatory and pollinating insects, resistance to insecticides, resurgence of secondary insect pests etc. Moreover, excessive use of such persistent insecticides on vegetables is acquiring a special concern since there is a little time lag between the last application and consumption. Owing to wide spectral problems with the use of these insecticides, use of bio-rational insecticides like plant origin insecticides is gaining popularity in Integrated Pest Management (IPM) because of their safety to non–targeted organisms and non-biomagnification in the food chain. Though, a variety of extracts from neem tree, *Azadirachta indica* have emerged as an important source of botanical insecticides which could control more than 105 insect pest species (SINGH *et al*., 1987 and OSMAN, 1993), so far, little research work has been carried–out to exploit other plant products having insecticidal properties. We have field evaluated of indigenous plant extracts against *P. xylostella* in cabbage crop.

MATERIAL AND METHODS

The field experiments were conducted during *Rabi*, 2009 to 2010 at the College of Agriculture, Central Agricultural University, Imphal, Manipur (India), to evaluate the comparative efficacy of nine aqueous indigenous plant extracts and one neem product (Nimbecidine) against *Plutells xylostella* Linnaeus in cabbage var. “Pride of India” under Imphal valley agro-ecological situations. The collected green parts of 20 plant samples were washed with distilled waters and made shade dry. The shade dried plant parts were ground in electrical grinder. Fifty gram of the ground materials of each plant sample was weighed and dissolved in distilled water to make a final volume of 1000 ml to which 1 gram of common soap was added. For all the experiments, seedlings were raised in properly prepared nursery beds. Before sowing, the seeds were treated with Saaf (Carbendazim 12% + Mencozeb 63%) 75 W.P. @ 2.00 gm/kg of seed in order to make the seeds disinfected from fungal diseases. The seedlings were ready for transplantation in the main field after 30 days of sowing. The locally recommended Agronomical practices were adopted for raising the experimental crops under study. The field experiments were laid out in randomized block design (RBD) with 11 (eleven) treatments including one untreated control each replicated three times. The 30 days old seedlings of test crop variety (Pride of India) were used for the experiment. The plot size, row to row and plant to plant spacing were 4 x 3 m^2, 45 cm and 45 cm, respectively with path of 1.0 m. Spray solution consisting of different insecticides in desired concentration was prepared separately for each treatment. All the spray treatments were applied by a high volume hand compression knapsack sprayer thrice at ten days intervals commencing from appearance of pest. The volume of the spray liquid was kept at 500 litres/ha. All the insecticides were applied in the evening hours. Care was taken at the time of spraying to avoid drifting of the insecticidal spray solution from one plot to another and to give a thorough coverage of the plants. Plain water was sprayed on the plants of untreated control plots. The relative field efficacy of the test insecticides was determined by recording the larval population of *P. xylostella* /plant at one day before and 1, 3, 5, 7 and 10 days after each application of insecticides from five randomly selected plants in each plot. The avoidable yield loss due to pests was computed by using formula suggested by PAWAR *et al*. (1984).

RESULTS AND DISCUSSION

The significant findings of the studies made during *Rabi,* 2009 – 2010 on the effect of intercropping on the evaluation of nine aqueous indigenous plant extracts and one commercial neem product (Nimbecidine) against diamond-back moth (DBM), *Plutella xylostella* Linnaeus in cabbage and effect of the insecticidal treatments on yield are briefly discussed below.

Effect on larval population of *P. xylostella*

The mean of two years' population (Table-1) revealed that the mean DBM population significantly differed among the treatments at 1,3,5,7 and 10 days after application in both the experimental years. Further, the results inflicted that Nimbecidine @ 2000 ml ha^{-1} proved to be the most effective with three sprays' mean larval population of 9.32 /plant as against 12.85 to 22.29 /plant in the rest aqueous plant extracts and 39.20/ plant in untreated check. Nimbecidine @ 2000 ml ha^{-1} resulted in maximum reduction of population (9.32 larvae/plant). It was closely followed by *Melia azedarach*, *Jatropha gossypifolia, Artemisia nilagirica, Lantana camara, Andropogon nardus* and *Ageratum conyzoides* each applied @ 12,500 ml ha^{-1} with their corresponding mean population of 12.85, 16.42, 18.04, 18.71, 18.84 and 18.88 larvae/plant, resopectively which differed significantly from the population observed in Nimbecidine treatment but, later four plant extracts had non significant difference from each other. Maximum mean larval population (22.29 larvae/plant) was recorded in plots treated with *Acorus calamus* extract @ 12,500 ml ha^{-1}. The effectiveness of Nimbecidine against DBM is being supported by the results of SAUCKE (1994b). On the other hand thec better performance of *J. gossypifolia* extract in checking the DBM population conforms to the findings of SINGH and KHUMAN (1995) who reported that out of seven indigenous extracts evaluated, *J. gossypifolia* at 1.0% concentration was found to be most effective against the pest. Results also showed that test plant extracts and commercial plant product provided satisfactory control of DBM, but proved inferior to Nimbecodine, *M. azedarach* and *J. gossypifolia* extract. The least effectiveness of these products might be due to their slow repellent/antifeedant/knock down action and lesser persistence.

Effect of the test insecticides on yield of cabbage

Yield of a crop is the interaction product of genetic potential of the variety, effect of prevailing environment and crop management practices including pest management adopted. It is usually expected that the treatments providing effective control of pests is to result in higher yield under a uniform ecological and crop management system. In the present investigation there was clear evidence that all the insecticidal treatments brought significant reduction of DBM population exhibited in significantly higher cabbage yield recording in insecticidal treatments in comparison to untreated control (14.35 t ha^{-1}) (Tables 2 and 3). Further, there was a general trend that the extent of yield increase was in accordance with the level of control of the pests. The more effective control of DBM by Nimbecidine, *M. azedarach*, *A. nilagirica*, *A. nardus*, *J. gossypifolia* and *M. koenigii* was manifested by higher production of yield of 21.18 to 61.01 per cent over untreated control. The plant derivatives having moderate effectiveness against the pests resulted in 18.11 to 19.60 tonnes per cent yield over control, the highest beings in *M. azedarach* treated plots. The other four plant extract treatments, *A. conyzoides*, *M. koenigii, L. camara* and *A. calamus* also increased yield to the extent of 21.18 to 35.54, respectively over untreated control. The highest mean yield (23.12 t ha^{-1}) was obtained from Nimbecidine treated plots followed by the plots treated with *M. azedarach* (21.98 t ha^{-1}), *A. nilagirica* (21.25 t ha^{-1}), *A. nardus* (20.22 t ha^{-1}), *J. gossypifolia* (19.85 t ha^{-1}) and *A. conyzoides* (19.45 t ha^{-1}) as against 17.39 to 18.30 t ha^{-1} in rest of the five insecticidal treatmented plots and 14.35 t ha^{-1} in untreated control.

Table-1: Effect of nine cow-urine indigenous plant extracts and Nimbecidine on larval population of diamond-back moth, *P. xylostella* in cabbage during *Rabi,*2009 and 2010

Treatment	**Dose (ml ha^{-1})**	**Mean larval population /plant after**			**Pooled Mean**	**DBS**	**Days after spraying**				
		1st spray	**2nd spray**	**3rd spray**			**1**	**3**	**5**	**7**	**10**
Melia azedarach	12,500	10.35 (3.43)	12.44 (3.21)	15.76 (4.19)	12.85 (3.61)	3.69 (2.04)	13.60 (3.75)	10.80 (3.35)	9.47 (3.15)	11.13 (3.40)	19.27 (4.41)
Murraya koenigii	12,500	16.79 (4.33)	19.66 (4.55)	25.38 (4.83)	20.61 (4.57)	3.98 (2.11)	20.73 (4.66)	13.27 (3.70)	18.27 (4.32)	24.87 (5.02)	25.93 (5.13)
Melothria perpusilla	12,500	17.12 (3.61)	19.85 (4.89)	26.15 (5.33)	21.04 (4.61)	4.16 (2.20)	16.87 (4.16)	17.80 (4.27)	18.33 (4.33)	24.47 (4.98)	27.90 (5.31)
Lantana camara	12,500	18.58 (4.32)	15.53 (3.98)	22.02 (4.66)	18.71 (4.32)	3.67 (2.06)	17.40 (4.22)	9.80 (3.20)	16.20 (4.08)	22.47 (4.78)	27.67 (5.30)
Acorus calamus	12,500	18.89 (4.34)	17.65 (4.45)	30.33 (5.40)	22.29 (4.73)	3.40 (1.96)	16.53 (4.12)	18.97 (4.40)	18.53 (4.36)	28.00 (5.33)	29.43 (5.46)
Jatropha gossypifolia	12,500	12.54 (3.24)	15.68 (3.89)	21.04 (5.08)	16.42 (4.07)	3.19 (1.91)	14.27 (3.83)	10.57 (3.32)	13.93 (3.79)	22.00 (4.73)	21.40 (4.67)
Ageratum conyzoides	12,500	17.55 (4.24)	16.24 (3.89)	22.85 (4.92)	18.88 (4.35)	3.98 (2.11)	17.50 (4.23)	10.03 (3.24)	18.33 (4.33)	22.20 (4.76)	26.33 (5.17)
Artemisia nilagirica	12,500	16.12 (4.04)	17.99 (4.43)	20.01 (4.31)	18.04 (4.26)	3.36 (2.03)	15.27 (3.97)	14.00 (3.80)	14.67 (3.88)	18.53 (4.36)	27.80 (5.31)
Andropogon nardus	12,500	16.58 (3.97)	18.11 (4.22)	21.83 (4.77)	18.84 (4.32)	3.98 (2.12)	14.33 (3.84)	14.67 (3.88)	14.73 (3.89)	20.13 (4.53)	29.33 (5.45)
Nimbecidine 0.03%	2,000	6.56 (2.55)	7.85 (2.98)	13.55 (3.74)	9.32 (3.09)	3.23 (1.91)	6.93 (2.72)	6.77 (2.66)	7.90 (2.88)	10.53 (3.32)	14.47 (3.86)
Control	Water	36.76 (5.46)	38.35 (6.12)	42.49 (6.60)	39.20 (6.06)	4.05 (2.14)	26.06 (5.13)	42.80 (6.56)	40.47 (6.39)	42.27 (5.53)	44.40 (6.69)
CD(P=0.05)		**0.38**	**1.01**	**0.71**	**0.46**	**NS**	**0.33**	**0.13**	**0.12**	**0.89**	**0.16**

Figures in parentheses are $\sqrt{X + 0.5}$ transformed values; DBS= Days before spraying;

[1]Composite means of five observations recorded at 1, 3, 5, 7 and 10 days after application;

[2]Mean of 3 replications based on 3 applications data

The cabbage yields recorded in all the insecticidal treatments were significantly higher than that of untreated control. The moderate effectiveness of *M. azedarach* and *J. gossypifolia* is supported by the findings of SINGH *et al.* (2004) who observed that among the plant extracts evaluated against the mustard aphid, *M. azedarach* and *J. gossypifolia* gave the best control registering higher cabbage yield. Considering the achievable yield of 23.12 t ha^{-1} with Nimbecidine treatment, the avoidable loss was computed ranging between 4.93% (*M. azedarach*) to 24.78% (*A. calamus*) in different insecticidal treatments and 37.93% in untreated control. Thus, there was a direct relationship between the level of pest control, cabbage yield and avoidable loss i.e., higher the treatment effective against the pest, more was the yield reflecting to lower available loss. The avoidable loss due to DBM, CB, and CA infestation varied from nil in Nimbecidine sprayed @ 2000 ml ha-1 to 37.93% in untreated check. Among the plots treated with insecticides maximum avoidable loss (24.78%) was computed in the plots treated with *A. calamus* extract @ 12500 ml ha^{-1}

The significant results generated from the present study carried out during *Rabi*, 2009 to 2010 revealed that *P. xylostella* is a regular pest of cabbage and maintained moderate level of population envisaging the need for their effective management. Even-though some of the aqueous indigenous plant extracts included in the test proved moderately effective against the pest and inferior to Nimbecidine, in consideration of indigenously available plant species to exploit their insecticidal properties it is advisable to use them. Therefore, cow-urine plant extracts *of M. azedarach*, *J. gossypifolia*, *A. nilagirica* and *A. nardus* may be incorporated in developing integrated *P. xylostella* management strategy under prevailing agro-climatic conditions of Manipur valley in order to obtain the optimum monetary benefit and causing minimum deterious effect of natural enemies of the pest.

Table-2: Overall effect of insecticides on the population of pests and yield of cabbage during November, 2006 – April, 2007

Treatment	**Dose** (ml ha^{-1})	**Mean larval population of *P.xylostella* /plant**	**Cabbage Yield (t ha^{-1})**	**Yield increase over control**	
				t ha^{-1}	**%**
Melia azedarach	12,500	12.85 (3.61)	21.98	7.63	53.17
Murraya koenigii	12,500	20.61 (4.57)	17.77	3.42	23.83
Melthria perpusilla	12,500	21.04 (4.61)	18.30	3.95	27.53
Lantana camara	12,500	18.71 (4.32)	17.45	3.10	21.60
Acorus calamus	12,500	22.29 (4.73)	17.39	3.04	21.18
Jatropha gossypifolia	12,500	16.42 (4.07)	19.85	5.50	38.33
Ageratum conyzoides	12,500	18.88 (4.35)	19.45	5.10	35.54
Artemisia nilagirica	12,500	18.04 (4.26)	21.25	6.90	48.08
Andropogon nardus	12,500	18.84(4.32)	20.22	5.87	40.91
Nimbecidine 0.03%	4000	9.32 (3.09)	23.12	8.77	61.11
Control	Water	39.20 (6.06)	14.35	-	-
CD (P= 0.05)		0.46	**0.82**	**-**	**-**

Figures in parentheses are $\sqrt{X + 0.5}$ transformed values;
[1]Mean larval/aphid population of six time intervals under observations based on 3 applications data

Table-3: Avoidable yield loss (%) of cabbage yield due to the three major insect pests and increase yield of different insecticidal treatments over control during Rabi-2009 to 2010

Treatments	**Dose**	**Mean of yield(t ha^{-1})**	**Avoidable yield loss per cent**
Melia azedarach	12,500 ml ha^{-1}	21.98	4.93
Murraya koenigii	12,500 ml ha^{-1}	17.77	23.14
Melthria perpusilla	12,500 ml ha^{-1}	18.30	20.85
Lantana camara	12,500 ml ha^{-1}	17.45	24.52
Acorus calamus	12,500 ml ha^{-1}	17.39	24.78
Jatropha gossypifolia	12,500 ml ha^{-1}	19.85	14.14
Ageratum conyzoides	12,500 ml ha^{-1}	19.45	15.87
Artemisia nilagirica	12,500 ml ha^{-1}	21.25	8.09
Andropogon nardus	12,500 ml ha^{-1}	20.22	12.54
Nimbecidine 0.03%	2000 ml ha^{-1}	23.12	0.00
Control		14.35	37.93

Note: Nimbecidine 0.03% @ 2000 ml ha^{-1} recorded the highest cabbage yield at 23.12 t ha^{-1} on the basis of which avoidable losses in untreated control (To) and in different insecticidal treatments have been determined.

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EFFECT OF CERTAIN IPM MODULES ON THE INCIDENCE OF YELLOW STEM BORER, GALL MIDGE AND LEAF FOLDER ON RICE UNDER IMPHAL AGRO-ECOLOGICAL SITUATIONS

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ABSTRACT: A field evaluation trial on the efficacy of three IPM modules (Bio-intensive module, Adoptive module and Insecticidal module) against *Scirpophaga incertulas* Walker, *Orseolia oryzae* Wood-Mason and *Cnaphalocrocis medinalis* Linnaeus was carried out in the farmer's field during *Kharif*, 2008 and 2009. Overall results of the investigation revealed that the adoptive module (M_2) recorded significantly lowest incidence of *S. incertulas* with mean dead heart(DH) and white ear head (WEH) of 1.14 and 2.44 per cent, respectively as against 5.34 and 8.34 per cent in farmer's practice (M_4), while insecticidal module was found to be most effective against both *O.oryzae* and *C.medinalis* registering minimum silver shoot (SS) and leaf damage (LD) incidence of 2.86 and 4.61 per cent, respectively followed by adoptive module (M_2) with 4.57% SS and 5.51% LD as against 8.36% SS and 13.45% LD in farmer's practice (M_4). The bio-intensive module (M_1) having no use of synthetic organic insecticides, although exhibited significantly better performance than the farmer's practice(check) did not perform well in reducing the incidence of these three major insect pests in comparison to the other two modules. The highest grain yield (6.12 t ha^{-1}) was recorded in the adoptive module treatment. It was closely followed by insecticidal module (5.16 t ha^{-1}) but had significant difference between them. The lowest grian yield (4.64 t ha $^{-1}$) was harvested from the plots treated with bio-intensive module. The avoidable yield loss was worked out to be 35.95 per cent in farmer's practice (check) which reduced to 15.69 (Insecticidal module) – 24.18 per cent (Bio-intensive module) in the IPM modules (other than adoptive module in which the avoidable loss has been taken as zero per cent).

Key words: IPM Modules, Efficacy, Rice, *Scirpophaga incertulas* Walker, *Orseolia oryzae* Wood-Mason and *Cnaphalocrocis medinalis* Linnaeus

INTRODUCTION

In Manipur, among the various insect pests inflicting loss to rice crop, yellow stem borer (YSB), *Scirpophaga incertulas* Walker, gall midge (GM), *Orseolia oryzae* Wood-Mason and leaffolder (LF), *Cnaphalocrocis medinalis* Linnaeus are considered as the major constraints in profitable cultivation of the crop. Large scale cultivation of high fertilizer responsive high yielding rice varieties coupled with assured perennial irrigation have increased the severity of these insect pests (PRAKASH *et al.*; 2014. However to combat these notorious phytophagous insects associated with rice, cultivators have their own system of pest control with various types of insecticides, particularly synthetic organic insecticides which are considered as the practical solution for intensive double rice crop cultivation. The effective control of various pests of rice by application of Monocrotophos has been suggested by many entomologists throughout the country (KRISHNAMURTHY *et al*., 1988; PANDA *et al*., 1989; MOHAN *et al*., 1991; SONTAKKE, 1993). But, due to high damage potential of the pests, problem of resistance development to most of the insecticides and high cost of insecticides it was considered imperative to field-evaluate a few IPM modules against these three major insect species

with a view to develop a strategy with rational use of insecticides in combination with other pest control components.

MATERIALS AND METHODS

Three IPM modules were field evaluated in a RBD trial on rice var., '**CAUR-1 (Tamphaphou)'** against YSB, GM & LF in farmer's field in the village Haorang Keirel of Imphal West District of Manipur during *Kharif*, 2008 and 2009. There were altogether four treatments including one check (Farmer's Practice), each replicated five times. The plot size was 1000 sq. m (50 m x 20 m). In order to assess the effectiveness of the treatments, observations were made at 30 and 50 days after transplanting (DAT) for GM, and 30, 50 and 90 DAT for stem borer and leaf folder. On each observation ten quadrates of one square metre each were taken in the field and the number tillers/leaves and dead heart (DH), white ear head (WEH), silver shoot (SS) and leaf damage (LD) were counted and computed in terms of percentage. Grain yield was recorded from different plots and finally converted to tonnes per hectare. The avoidable yield loss (%) was determined for different treatments using the formula suggested by PAWAR *et al*. (1984). The data obtained from the experiment were subjected to statistical analysis after suitable transformations wherever necessary.

Details of treatments:

M_1=	(Bio-intensive Module)	: Soaking of seeds in 0.5% solution of Neemcel (Azadirachtin 10000 ppm) for 6 hours + Judicious use of NPK @ 60:40:30 kg ha^{-1} + foliar spray with Neemcel @ 750 ml ha^{-1} at 20 DAT + Release of *Trichogramma chilonis* @ 50,000 eggs/ha/week for two weeks starting from 30 DAT + spray with Neemcel @ 750 ml ha^{-1} at panicle initiation stage.
M_2=	(Adoptive Module)	: Sprouted seed treatment with 0.2% solution of Chlorpyrophos 20 EC for 3 hours + Judicious use of NPK @ 60:40:30 kg ha^{-1} + foliar spray with Imidacloprid 17.8 SL @ 20 g a.i. ha^{-1} at 20 DAT + Installation of Pheromone trap for *S. incertulas* @ 15 traps ha^{-1} at 30 DAT+ spray with Imidacloprid 17.8 SL @ 20 g a.i. ha^{-1} at panicle initiation stage.
M_3=	(Insecticidal Module)	: Soaking of seeds in 0.2% solution of Monocrotophos 36 WSC for 6 hours + Judicious use of NPK @ 60:40:30 kg ha^{-1} + foliar spray with Monocrotophos @ 500 g a.i. ha^{-1} at 20, 45 and 75 DAT
M_4=	(Farmer's Practice)	: Check

RESULTS AND DISCUSSION

The pooled data generated from the present field study conducted during *Kharif* season of 2008 and 2009 revealed that all the three IPM modules were significantly superior to check treatment (Farmer's Practice) in respect of insect control and grain yield (Table-1). Incidence of stem borer causing DH and WEH in different IPM modules varied from 1.14 – 2.62 and 2.44 – 3.66 per cent, respectively as against 5.34 and 8.34 per cent, respectively in farmer's practice (M_4). The adoptive module (M_2) recorded the lowest incidence of DH (1.14 %) and WEH (2.44%) followed by insecticidal module with a record of mean DH and WEH of 1.88 and 3.66%, respectively but had significant difference between them. Amongst the three modules, the highest stem borer infestation (2.62% DH & 4.26% WEH) was registered in bio-intensive module (M_1). As regards gall midge infestation, the silver shoot incidence ranged between 2.86 to 5.72% in three IPM modules as against 8.36 per cent in farmer's practice (M_4). The minimum SS incidence

(2.86%) was observed in insecticidal module (M_3) followed by adoptive module (4.72%). The bio-intensive module was quite ineffective in suppression of gall midge infestation with maximum incidence of 5.72% SS. As evident from Table-1, leaf folder (LF) incidence in all the IPM modules also differed significantly from one another. However, the insecticidal module (M_3) registered the lowest DL (4.61%) as against 13.45% in farmer's practice (M_4), closely followed by adoptive module (M_2) recording mean DL of 5.51%, but had significant difference between them. The bio-intensive module (M_1) having no use of synthetic organic insecticides, although was significantly better than the farmer's practice (check) did not perform well in compared with the other two modules.

IPM modules evaluated against these major rice insect-pests have not been tested by earlier workers. Therefore, the results obtained here on this aspect could not be substantiated. However, the effectiveness of insecticidal module (M_3) against *S. incertulas* is partially in accordance with the results of RAJAMANI (1985) and PANDA *et al*.(1989) who recorded the effective control of stem borer (DH) and plant hoppers and higher grain yield due to the application of Monocrotophos 36 WSC at 0.05% spray concentration at 50 and 75 days after planting.

Table-1: Relative field efficacy of various IPM modules against *S. incertulas*, *O. oryzae* and *C. medinalis* on rice var. 'CAUR-1 during *Kharif*, 2008 and 2009

Treatment	**[1]Mean pest infestation of two years in different treatments**				**[2]Mean grain yield (t ha^{-1})**
	S. incertulas		***O. oryzae***	***C. medinalis***	
	DH (%)	**WEH (%)**	**SS (%)**	**DL (%)**	
M_1=Bio-intensive Module	2.62 (9.25)	4.26 (11.91)	5.72 (13.71)	6.68 (14.89)	4.64
M_2=Adoptive Module	1.14 (6.00)	2.44 (8.44)	4.57 (12.26)	5.51 (13.54)	6.12
M_3= Insecticidal Module	1.88 (7.81)	3.66 (11.03)	2.86 (9.53)	4.61 (12.34)	5.16
M_4= Farmer's Practice	5.34 (13.28)	8.34 (16.78)	8.36 (16.93)	13.45 (21.43)	3.92
SE(m) ±	**0.10**	**0.17**	**0.19**	**0.14**	**0.10**
CD (P= 0.05)	**0.26**	**0.43**	**0.47**	**0.36**	**0.25**

Figures in the parentheses are angular transformed values; [1]Mean of two (*O. oryzae*) and three (*S. incertulas & C. medinalis*) observation periods based on five replications; [2]Mean of five replications based on two consecutive experimental years

The mean grain yield in different IPM modules varied between 4.64 (bio-intensive module) to 6.12 t ha^{-1} (adoptive module) as against 3.92 t ha^{-1} in check (farmer's practice). Application of different IPM modules resulted in increase of 0.72 t ha $^{-1}$ (18.37%) to 2.20 t ha^{-1} (56.12%) grain yield over farmer's practice (Table-2). The avoidable yield loss was worked out to be 35.95% in farmer's practice (check) which reduced to 15.69-24.18% in two IPM modules (other than adoptive module in which the avoidable loss has been taken as zero per cent). In consideration of environmental safety in one hand and effective management of the pests with reasonable cost effectiveness, it is advisable to adopt the management strategy involving bio-control agents/pheromone

with limited use of synthetic chemical insecticides instead of solely depending upon bio-control agents/ pheromone or chemicals.

Table-2: Avoidable yield losses (%) of different treatments in *O. oryzae, S. incertulas & C. medinalis* control on rice var. 'CAUR-1' during *kharif* season of 2008 and 2009

Treatment	Mean grain Yield (t ha^{-1})	Increase of yield over check i.e. Farmer's Practice (FP)		Avoidable loss (%)
		Tonnes/ha	Per cent	
M_1=Bio-intensive Module	4.64	0.72	18.37	24.18
M_2=Adoptive Module	6.12	2.20	56.12	0.00
M_3= Insecticidal Module	5.16	1.24	31.63	15.69
M_4= Farmer's Practice (Check)	3.92	-	-	35.95

M_2 (Adoptive Module) recorded the maximum grain yield of 6.12 t ha^{-1} on the basis of which avoidable losses in check (FP) and in different treatments have been computed

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BIOEFFICACY OF NOVALURON 5.25% + INDOXACARB 4.5% SC AGAINST PIGEONPEA POD BORER COMPLEX

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ABSTRACT: Experiment was conducted to study the bio-efficacy of Combination product (Novaluron 5.25%+Indoxacarb 4.5% SC) against pod borer complex in pigeonpea ecosystem for two seasons in the year 2012-13 and 2013-14. Significantly lowest number of pod fly population was recorded in the treatment with combi product @875 ml/ha (1.50 and 1.91 Pod fly/5 plants in 2012-13 and 2013-14, respectively) ten days after the treatments. Similarly pod borer population was significantly lowest in the treatment combi product @875 ml/ha with 1.62 and 1.80 pod borer/ 5 plants in 2012-13 and 2013-14, respectively. Least pod damage was recorded in the treatment Novaluron 5.25% + Indoxacarb 4.5% SC at 875 ml/ha (2.44% in 2012-13 and 2.08% in 2013-14). However highest dry pod yield was recorded in the treatment Novaluron 5.25% + Indoxacarb 4.5% SC with 20.00 and 19.20 q/ha in the year 2012-13 and 2013-14, respectively, hence Novaluron 5.25% + Indoxacarb 4.5% SC commination found to be very effective in controlling the pod borer complex in pigeonpea ecosystem.

Key words: Novaluron, Indoxacarb, bioefficacy, pod borer, pigeonpea, pod fly

INTRODUCTION

Pigeonpea, *Cajanus cajan* (Linn.), commonly known as redgram, tur, arhar etc., is an erect and short-lived perennial shrub legume. India accounts for about 75 % of world production. Economically it is the second most important pulse crop after chickpea in India accounting for about 20% of total pulse production. Its productivity is about 806 kg /ha in India (ANONYMOUS, 2012). The yield of pigeonpea is not satisfactory as compared to area under cultivation. Among many constraints for lower grain yield of pigeonpea in India, attack of insect pest is most important. More than 200 species of insects are recorded as pests of pigeonpea (LATEEF and REED, 1980). Among the insect species, pod borer complex is reported to reduce the yield upto 27.77%, particularly gram pod borer (*Helicoverpa armigera* Hubner) is considered as most destructive. The important pests which infest pigeonpea are gram pod borer (*Helicoverpa armigera* Hub), plume moth (*Exelastis atomosa* Wall.), pod fly (*Melanagromyza obtuse* Mall) and legume pod borer, *Maruca vitrata*. Chemical insecticides are generally preferred for the control of pest due to their easy availability and applicability, but their excessive and indiscriminate use has resulted in plethora of problems e.g. resurgence of minor insect pests, insecticidal resistance in insects, mortality of natural enemies and non target species and pesticide residue in harvested produce leading to various health hazards, besides the increased cost of cultivation per unit area. To overcome these problems, it has now become imperative to select safer insecticides and combination products that should protect the crop and keep the pest population below injury level. Number of combination products has been registered and available in the market to control the insect pests in field crops. Reports are also available that they have been found effective. With this background, field experiment was conducted to know the efficacy of combi product Novaluron 5.25% +Indoxacarb 4.5% SC against pigeonpea pod borer complex.

MATERIAL AND METHODS

Field trial was carried out for the evaluation of combi product (Novaluron 5.25% +Indoxacarb 4.5% SC) against pigeonpea pest in irrigated area kharif 2012-13 and kharif 2013-14. The experiment was laid out in a Randomized Block Design (RBD). The variety was used BSMR-736 with inter row spacing of 90cm and intra row spacing of 30cm. The recommended dose of fertilizers was applied to maintain good plant stand through the crop period. The plot size for each treatment was 5.0X5.0 m with replications. The combi product was evaluated at three doses of 750,825,875 ml/ha and compared with standard insecticides, Novaluron 10% EC @750ml/ha, Indoxacarb 14.5% SC @400ml/ha and Lambda–cyhalothrin 5% EC @ 500 ml/ha and compared with untreated control. During study period, three sprays were given.

Observations were recorded at day before spray, 1st, 5th, 7th and 10th days after each application on pod fly (*Melonagromyza abtusa*) and gram pod borer (*Helicoverpa armigera*) survived larval population on five randomly selected and tagged plants on top five pods at each spray. Number of survived larvae was noted on these randomly selected plants. Average survived larval population during the period of three spray applications at ten days interval recorded per plant in various treatments were worked out in square root transformation subjected to statistical analysis. Per cent fruit damage was recorded on the basis of fruit yield. Dry pods were harvested at harvest, shade dried thoroughly and weight per treatment per replication was recorded. Later yield per plot was worked out and it is converted to per ha basis and subjected to statistical analysis.

RESULTS AND DISCUSSION

Pod fly (*Melonagromyza abtusa*) population

Pod fly population was found to be non significant day before application of the treatment, it was ranged from 3.86 to 4.32 pod fly / 5 plants in the year 2012-13, whereas in the year 2013-14, pod fly population was ranged from 4.88 to 5.52 Pod fly population. In 2012-13 significantly lowest number of pod fly population was recorded in the treatment with combi product @875 ml/ha (1.5 Pod fly / 5 plants) ten days after the treatments, which was on par with combi product @825 ml/ha (1.54 Pod fly / 5 plants). The remaining treatment recorded pod fly population ranged from 2.45 to 3.36 pod fly / 5 plants, whereas in 2013-14, pod fly population was significantly lowest in the treatment with combi product @875 ml/ha with 1.91 Pod fly / 5 plants ten days after the application. This treatment was statistically no par with the treatment combi product @825 ml/ha (1.96 Pod fly / 5 plants). Highest pod fly population was recorded in the treatment 10.5 pod fly / 5 plants

The results are in line with reports of DAS *et al.*(2015), tested the effect of different insecticides and their mix formulations (Novaluron 10 EC, Indoxacarb 14.5SC, Emamectin benzoate 5SG, Lambda-cyhalothrin 5EC, Thiamethoxam 25WG, Fipronil 5SC, Novaluron 5.25+Indoxacarb 4.5SC, Novaluron 5.25+fipronil 4SC and Indoxacarb 14.5SC+Thiamethoxam 25WG) against pigeon pea pod borer and pod fly. Mixed formulation of Novaluron 5.25+Indoxacarb 4.5SC @ 80 g a.i./ha and Novaluron 5.25+Fipronil 4SC @ 80 g a.i./ha, were found to be the most effective with cent percent reduction of *Helicoverpa* larval population, whereas in case of pod fly, Novaluron +

Table-1: Bio-efficacy of Novaluron 5.25% + Indoxacarb 4.5% SC against pod fly (*Melonagromyza abtusa*)

S. No	Treatment	Dosage (ml/ha)	Avg. population of Pod fly / 5 plants (III Spraying-kharif (2012-13)						Avg. population of Pod fly / 5 plants (III Spraying-kharif (2013-14)					
			DBA	1 DAA	5 DAA	7 DAA	10 DAA	Mean	DBA	1 DAA	5 DAA	7 DAA	10 DAA	Mean
1	Novaluron 5.25% + Indoxacarb 4.5% SC*	750	4.32 (2.20)	3.89 (1.97)	3.62 (1.9)	3.52 (1.88)	3.36 (1.83)	3.59	5.52 (2.45)	4.95 (2.22)	4.63 (2.15)	4.84 (2.20)	4.30 (2.07	4.68
2	Novaluron 5.25% + Indoxacarb 4.5% SC*	825	3.90 (2.10)	2.78 (1.67)	2.31 (1.52)	2.12 (1.46)	1.54 (1.24)	2.91	5.00 (2.35)	3.55 (1.88)	2.85 (1.69)	2.56 (1.60)	1.96 (1.40)	2.73
3	Novaluron 5.25% + Indoxacarb 4.5% SC*	875	3.86 (2.09)	2.73 (1.65)	2.21 (1.49)	1.71 (1.31)	1.5 (1.22)	2.01	4.88 (2.32)	3.49 (1.87)	2.74 (1.66)	2.08 (1.44)	1.91 (1.38)	2.55
4	Novaluron 10 % EC	750	4.14 (2.15)	3.47 (1.86)	3.10 (1.76)	2.83 (1.68)	2.61 (1.62)	2.51	5.28 (2.40)	4.44 (2.11)	3.97 (1.99)	3.58 (1.89)	3.33 (1.82)	3.83
5	Indoxacarb 14.5% SC	400	4.00 (2.12)	3.34 (1.83)	3.02 (1.74)	2.75 (1.66)	2.45 (1.57)	2.89	5.14 (2.37)	4.27 (2.07)	3.87 (1.97	3.47 (1.86)	3.13 (1.77)	3.68
6	Lambda-Cyhalothrin 5% EC	500	4.20 (2.17)	3.59 (1.89)	3.16 (1.78)	3.15 (1.77)	2.85 (1.69)	3.18	5.36 (2.42)	4.59 (2.14)	4.15 (2.04)	3.91 (1.98)	3.64 (1.91)	4.07
7	Untreated control	--	4.24 (2.18)	6.86 (2.62)	7.48 (2.73)	7.81 (2.79)	8.21 (2.87)	7.59	5.42 (2.43)	8.75 (2.96)	9.57 (3.09)	10.0 (3.16)	10.5 (3.24)	9.70
S.Em ±			**0.14**	**0.04**	**0.06**	**0.06**	**0.07**		**0.16**	**0.05**	**0.06**	**0.07**	**0.09**	
CD (P=0.05)			**NS**	**0.13**	**0.21**	**0.18**	**0.22**		**NS**	**0.15**	**0.20**	**0.23**	**0.28**	

Fipronil @ 80 g a.i./ha and Fipronil @ 50 g a.i./ha were the best with 72.5% reduction of pod fly population for each treatment.

Gram pod borer (*Helicoverpa armigera*) Population:
Results of the investigation revealed that mean population of gram pod borer (*Helicoverpa armigera***)** ranged from 2.37 to 8.23 and 2.03 to 9.21 in kharif during 2012-13 and 2013-14, respectively. Gram pod borer population in all the treatments is significantly lower than untreated control (8.23 and 9.21 pod borer /5plant). In 2012-13 pod borer population was significantly lowest in the treatment with combi product @875 ml/ha (1.62 pod borer/ 5 plants) ten days after the spray. This was on par with treatment with combi product @825 ml/ha (1.82 pod borer/5 plants). Remaining treatment recorded the pod borer population ranging from 3.19 to 4.14 pod borer/ 5 plants. In the untreated control the pod borer population was recorded highest with 8.87 pod borer/5 plants. Similar trend was observed in the year 2013-14, with significantly lowest pod borer population recorded in treatment combi product @875 ml/ha (1.80 pod borer/5 plants).

The results are in line with the reports of GOSHAL *et al*. (2016) who studied the effect of different treatment schedules of Novaluron 5.25% + Indoxacarb 4.5% SC against pod borer of pigeon pea gave superlative effect over the sole insecticide Novaluron and Indoxacarb and standard check Lamda-cyhalothrin. Among the three selected dose of Novaluron 5.25% + Indoxacarb 4.5% SC @ 875 ml/ha was recorded as best in managing *H. armigera* population up to harvesting period (mean 0.03% infested pod of both years), while, @ 825 ml/ha also recorded remarkable effect on the target pest. Similarly BABARIYA (2010) evaluated the different insecticides against gram pod borer, *H. armigera* infesting pigeonpea. Results revealed that the insecticide Indoxacarb 0.0075% gave the highest per cent mortality of the pest followed by Spinosad 0.009%, Profenophos + Cypermethrin 0.044% and Endosulfan 0.07%.

However different insecticides were evaluated against legume pod borer, *Maruca vitrata* and *H. armigera* in pigeonpea and found that, inflorescence damage due to Maruca was lowest in Chlorantraniliprole 18.5 SC (2.08%) and Flubendiamide 39.35 SC (3.64%), followed by Spinosad 45 SC (6.21%) as against control (31.18%) with 93.3, 88.3 and 80.1% reduction over control respectively as reported by SREEKANTH *et al.* (2015b).

Pigeonpea dry pod yield and per cent pod damage:
Dry pod yield harvested from different treatments varied greatly showing the impact and effectiveness of treatments. The maximum pod yield recorded under the chemical treatment Novaluron 5.25% + Indoxacarb 4.5% SC at 875 ml/ha (20.00 q/ha in 2012-13 and 19.20 q/ha in 2013-14) and it was found at par with treatment Novaluron 5.25% + Indoxacarb 4.5% SC at 825 ml/ha (19.90 q/ha in 2012-13 and 19.10 q/ha in 2013-14). However, all the above treatments were significantly superior over rest of the treatments. Untreated control treatment recorded very less dry pod yield (16.00 q/ha in 2012-13 and 15.40 q/ha in 2013-14) which clearly depicts that if pigeon pea crop is not protected properly at right time with right molecule more than 60% loss is expected (Table-3).

Table-2: Bio-efficacy of Novaluron 5.25% + Indoxacarb 4.5% SC against gram pod borer (*Helicoverpa armigera*)

S. No	Treatment details	Dosage (ml/ha)	Avg. population of Gram pod borer / 5 plants (III Spraying- kharif (2012-13)						Avg. population of Gram pod borer / 5 plants (III Spraying- kharif (2013-14)					
			DBA	1 DAA	5 DAA	7 DAA	10 DAA	Mean	DBA	1 DAA	5 DAA	7 DAA	10 DAA	Mean
1	Novaluron 5.25% + Indoxacarb 4.5% SC*	750	5.22 (2.39)	4.75 (2.18)	4.52 (2.13)	4.36 (2.09)	4.14 (2.03)	4.44	5.84 (2.52)	5.33 (2.31)	5.04 (2.24)	4.87 (2.21)	4.63 (2.15)	4.96
2	Novaluron 5.25% + Indoxacarb 4.5% SC*	825	4.86 (2.32)	3.27 (1.81)	2.69 (1.64)	2.18 (1.48)	1.82 (1.35)	2.49	5.40 (2.43)	3.66 (1.91)	3.01 (1.73)	2.44 (1.56)	2.02 (1.42)	2.03
3	Novaluron 5.25% + Indoxacarb 4.5% SC*	875	4.78 (2.30)	3.18 (1.78)	2.60 (1.61)	2.08 (1.44)	1.62 (1.27)	2.37	5.36 (2.42)	3.54 (1.88)	2.91 (1.71)	2.32 (1.52)	1.80 (1.34)	2.64
4	Novaluron 10 % EC	750	5.10 (2.37)	4.14 (2.03)	3.81 (1.95)	3.53 (1.88)	3.36 (1.83)	3.71	5.64 (2.48)	4.64 (2.15)	4.26 (2.06)	3.95 (1.99)	3.73 (1.93)	4.14
5	Indoxacarb 14.5% SC	400	5.00 (2.35)	4.02 (2.00)	3.73 (1.93)	3.41 (1.85)	3.19 (1.79)	3.58	5.60 (2.47)	4.51 (2.12)	4.17 (2.04)	3.81 (1.95)	3.57 (1.89)	4.01
6	Lambda-Cyhalothrin 5% EC	500	5.12 (2.37)	4.39 (2.10)	4.10 (2.02)	4.01 (2.00)	3.72 (1.93)	4.05	5.72 (2.49)	4.92 (2.22)	4.59 (2.14)	4.49 (2.12)	4.13 (2.03)	4.53
7	Untreated control	--	5.16 (2.38)	7.53 (2.74)	8.05 (2.84)	8.48 (2.91)	8.87 (2.98)	8.23	5.77 (2.50)	8.43 (2.90)	9.01 (3.00)	9.49 (3.08)	9.93 (3.15)	9.21
S.Em ±			**0.11**	**0.06**	**0.06**	**0.10**	**0.1**		**0.13**	**0.06**	**0.06**	**0.09**	**0.08**	
CD (P=0.05)			**NS**	**0.18**	**0.20**	**0.31**	**0.30**		**NS**	**0.2**	**0.19**	**0.27**	**0.27**	

Similarly, the least pod damages were recorded under treatment Novaluron 5.25% + Indoxacarb 4.5% SC at 875 ml/ha (2.44% in 2012-13 and 2.08% in 2013-14), which was found to be on par with chemical trématent Novaluron 5.25% + Indoxacarb 4.5% SC at 825 ml/ha (2.53% in 2012-13 and 2.16% in 2013-14). The maximum pod damage was noticed in untreated control (14.70% in 2012-13 and 12.57% in 2013-14) (Table-3).

The results agreed to the reports of KATTI and SURPUR (2015) evaluated the efficacy of Novaluron 5.25%+ Indoxacarb 4.5% SC (MAIRM-01 SC) against *Spodoptera litura* and *H. armigera* on tomato. Novaluron 5.25%+indoxacarb 4.5% SC (MAIRM-01 SC) was tested @ 750, 825,875 ml/ha along with Novaluron @ 750 ml/ha, Indoxacarb @ 500 ml/ha and Lambda cyhalothrin @ 300 ml/ha were kept as standard checks. The results revealed that Novaluron 10 EC @ 750 ml/ha and Indoxacarb 14.5% SC @ 500 ml/ha were significantly superior in managing *S. litura* and *H. armigera* over rest of the treatments. Different insecticides were tested against gram pod borer in pigeonpea and results revealed that Indoxacarb 0.007 per cent, Spinosad 0.005 per cent and Emamectine benzoate 0.005 per cent were found to be the most effective in reducing the gram pod borer population as reported by KHORASIYA (2014). Similarly SREEKANTH *et al.* (2015a), who evaluated different insecticides against *H. armigera* in pigeonpea ecosystem and found that pod damage was lowest (5.9%) in plots treated with Acetamiprid and Dimethoate, followed by Fipronil (6.6%) and Thiamethoxam (7.2%) with 42.2, 42.2, 35.3 and 29.4 per cent reduction over control, respectively.

Table-3: Effect of Novaluron 5.25% + Indoxacarb 4.5% SC on % pod damage & pod yield

S. No.	**Treatments**		**Kharif (2012-13)**		**Kharif (2013-14)**	
		Dosage (ml/ha)	**% pod Damage**	**Dry pod yield (q/ha)**	**% pod Damage**	**Dry pod yield (q/ha)**
1	Novaluron 5.25% + Indoxacarb 4.5% SC	750	5.92 (14.08)*	17.58	5.00 (12.92)*	16.87
2	Novaluron 5.25% + Indoxacarb 4.5% SC	825	2.53 (9.15)	19.90	2.16 (8.45)	19.10
3	Novaluron 5.25% + Indoxacarb 4.5% SC	875	2.44 (8.99)	20.00	2.08 (8.29)	19.20
4	Novaluron 10 % EC	750	3.00 (9.97)	19.32	2.56 (9.21)	18.54
5	Indoxacarb 14.5% SC	400	3.80 (11.24)	18.15	3.25 (10.39)	17.42
6	Lambda-Cyhalothrin 5% EC	500	4.46 (12.19)	16.45	3.80 (11.24)	15.79
7	Untreated control	---	14.70 (22.54)	16.00	12.57 (20.77)	15.40
S. Em ±			0.13	0.13	0.14	0.17
CD (P=0.05)			0.41	0.40	0.43	0.52

DAS *et al.*(2015) reported highest yield was recorded in Novaluron+Fipronil @ 80 g a.i./ha treated plot (18.6 q/ha) followed by Novaluron+Indoxacarb and Fipronil treated plot with 16.4 and 16.2 q/ha, respectively while in control plot it was only 7.5q/ha. Similarly BABARIYA (2010) noticed that the insecticide Indoxacarb 0.0075% recorded significantly highest grain yield (1486 kg/ha). Similarly KHORASIYA (2014) also reported highest yield was recorded in the treatment of Indoxacarb 0.007% (1658 kg/ ha) followed by Spinosad 0.005% (1582 kg/ha) and Emamectine benzoate 0.005% (1494 kg/ha).

The maximum yield (23.40 q/ha) was obtained from the plots treated with Novaluron 5.25% + Indoxacarb 4.5% SC @ 875 ml/ha closely followed by @ 825 ml/ha (22.98 q/ha) reported by GOSHAL *et al*., (2016). KATTI and SURPUR (2015) reported that Novaluron 5.25+Indoxacarb 4.5% @ 875 ml/ha recorded lowest fruit damage and higher fruit yield.

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BIO-EFFICACY OF CERTAIN NEWER INSECTICIDES AGAINST COTTON BOLLWORM UNDER HIGH DENSITY PLANTING SYSTEM

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ABSTRACT: Field experiments were conducted at the experimental field of Dept. of Entomology, College of Agriculture, Badnapur during *Kharif* season of 2015 – 16to evaluate the efficacy of newer insecticides against cotton bollworms under high density planting system. The treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha proved 4 DAS. The rest of all the treatments were also significantly superior over control except profenophos 50 EC @ 1500 ml/ha after both 1st and 2nd spray. The data recorded in relation to green boll damage after 1stand 2nd spray at 1, 3, 7, 10 and 14 DAS the treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha proved significantly superior with 1.33, 1.55, 1.53, 1.18 and 1.67 per cent damage while profenophos 50 EC @ 1500 ml/ha proved least effective treatment with 2.48, 2.94, 2.92, 2.71 and 3.27 per cent damage respectively. The treatment of emamectin benzoate 5 SG @ 220 gm/ha recorded highest yield *i.e.* 777 kg/ha but it was statistically at par with rest of the six treatments.

Key words: Cotton, Efficacy, Newerinsecticides,Bollworms, HDPS

INTRODUCTION

Cotton is of tropical origin but is most successfully cultivated in temperate climates with well-distributed rainfall. India is second largest producer of cotton in the world after China. High density planting system are commonly followed to obtained high yield with straight varieties across the world especially in the major cotton growing countries such as U.S.A., Australia, China, Brazil and Uzbekistan. The planting geometry is 8-10 cm distance between plants in a row with row to row distance at 18, 30, 45, 60, 75, 90 and 100 cm. The planting methods are referred as narrow row if the row to row spacing is less than 75 cm and ultra narrow row if the spacing is less than 45 cm. Generally wider row to row spacing is followed in deep soil and irrigated farms and ultra narrow row spacing in rainfed condition (ANONYMOUS, 2014).

Cotton Bollworm is the most serious pest of cotton. Damage to cotton plants occurs through the feeding of the larvae (caterpillars). Larvae feed on the new growth, developing buds and bolls and even large bolls. Damage can be serious with total loss of bolls in heavily infested plants. Yield losses due to bollworms in Ghana in the absence of an efficient management intervention range from 39 to 55 per cent. Higher yield can be realized only if the pest populations can be kept below the economic injury levels (KRANTHI *et al*., 2002). Generally three major bollworms are attacked the cotton crop, together called bollworm complex includes american bollworm *Helicoverpa armigera,* spotted bollworm *Earias insulana,* pink bollworm *Pectinophora gossypiella.* Cotton bollworm is often difficult to control with insecticides due to its ability to develop resistance against frequently used insecticides. Therefore, the efforts are made to find out the effective new molecules of insecticides for management of bollworm complex in cotton under high density planting system.

MATERIALS AND METHODS

Field experiments were conducted at the experimental field of Dept. of Entomology, College of Agriculture, Badnapur during *Kharif* season of 2015-16, under randomized block design.Cotton variety NH-615 (Desi cotton) was sown under high density planting system on 31st July 2015 in a plot size of 4.5m×5.0m with a spacing of 45x10cm. Other agronomic practices were followed as per local recommendation. There were eight treatments comprising of Emamectin benzoate 5 SG, Lambda cyhalothrin 5 EC, Chlorantraniliprole 18.5 SC, Indoxacarb 15.8 SC, Spinosad 45 SC, Fenvalerate 20 SC, Profenophos 50 EC, including control (Table-1). The ETL of bollworms was observed before spraying. The larval population FBD and open boll damage of cotton bollworms was counted on randomly selected five plants at weekly interval till crop harvest. The first spray was initiated at ETL. Observation of cotton bollworms were recorded five randomly selected plants of each plot. Observations were recorded from 24 hours prior to spraying and subsequently, post treatment counts were recorded after 1, 3, 7, 10 and 14 days of spraying, respectively. All the data was subjected to statistical analysis after appropriate transformation as suggested by GOMEZ and GOMEZ, 1984.

RESULTS AND DISCUSSION

Population of *Helicoverpa*

The data on *Helicoverpa* population **one day before first spray** (Table-1) showed statistically non significant differences indicating uniform distribution of bollworm population. Further, one day after first spray, all the treatments recorded significant lower (1.53 to 2.68 larvae/ plant) than untreated (5.67 larvae/plant) control. The chlorantraniliprole 18.5 SC @ 150 ml/ha, recorded least population (1.53 larvae/plant), which was significantly superior and at par with rest of the treatments except profenophos 50 EC @ 1500 ml/ha which was stand second best treatment and proved superior over control. Three days after first spray, all the treatments recorded lower (1.53 to 4.33 larvae/plant) population of *Helicoverpa* than untreated (5.5 larvae/plant) control.Thetreatment of chlorantraniliprole 18.5 SC @ 150 ml/ha (1.53 larvae/plant), which was significantly superior and at par with emamectin benzoate 5 SG @ 220 gm/ha and lambda cyhalothrin 5 EC @ 250 ml/ha and rest of the treatments were exhibited the poor performance.

Seven days after first spray, all the treatments recorded the lowest population in the range (2.25 to 2.98 larvae/plant) and highest in untreated control (4.46 larvae/plant).The treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded least population of *H. armigera* (2.25larvae/plant), which was significantly superior over control and at par with emamectin benzoate 5 SG @ 220 gm/ha, lambda cyhalothrin 5 EC @ 250 ml/ha, indoxacarb 15.8 EC @ 250 ml/ha and spinosad 45 SC @ 125 ml/ha except profenophos 50 EC @ 1500 ml/ha which was stands second best treatment and proved superior over untreated control.

Fourteen days after first spray, all the treatments recorded significantly lower (2.60 to 6.90 larvae/ plant) than untreated (8.24 larvae/ plant).The treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded least population of *H. armigera* (2.60 larvae/plant) and was par with fenvalerate 20 EC (2.93 larvae/plant) @ 400 ml/ha, emamectin benzoate 5 SG @ 220 mg/ha and lambda cyhalothrin 5 EC @ 250 ml/ha ml while spinosad 45 SC @ 125 ml/ha, indoxacarb 15.8 EC @ 250 ml/ha and profenophos 50 EC @ 1500 ml/ha were the secondly superior over control.

Population of *Helicoverpa* after second spray

The data on *Helicoverpa* population on one day before second spray presented (Table-1) were statistically non significant indicating uniform distribution of bollworm population. One day after second spray, all the treatments recorded significantly lower (1.59 to 4.86 larvae/plant) population of *Helicoverpa* and maximum (6.30 larvae/plant) in control.The treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded least population of *Helicoverpa* (1.59 larvae/plant), which were proved better and was at par with emamectin benzoate 5 SG @ 220 gm/ha, lambda cyhalothrin 5 EC @ 250 ml/ha. The treatment of indoxacarb 15.8 EC @ 250 ml/ha, spinosad 45 SC @ 125 gm/ha and fenvalerate 20 SC @ 400 ml/ha, were the statistically second best treatment while profenophos 50 EC @ 1500 ml/ha exhibit the poor performance.

Three days after second spray, the suppression of pest observed in the range of (2.16 to 5.73 larvae/plant) as compare to (9.54 larvae/plant) in untreated control.The treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded least population of *H. armigera* (2.16 larvae/plant), which was at par with emamectin benzoate 5 SG @ 220 gm/ha and lambda cyhalothrin 5 EC @ 250 ml/ha while rest of the treatments were stand statistically second best treatments over control. Seven days after second spray, the range of *Helicoverpa* in all the treated plots were (2.0 to 3.84 larvae/plant) against (5.73 larvae/plant) in untreated plots.The treatment of indoxacarb 15.8 EC @ 250 ml/ha recorded least population of *H. armigera* (2.0 larvae/plant), and at par with rest of the treatments. Ten days after second spray, all the treatments recorded significantly lower (1.53 to 3.84 larvae/plant) against (6.20 larvae/plant) in untreated control.The treatment of indoxacarb 15.8 EC @ 250 ml/ha was significantly superior over control (1.53 larvae/plant) and which was statistically at par with rest of the treatments except profenophos 50 EC @ 1500 ml/ha which was secondly superior over control.

Fourteen days after second spray, the population of *Helicoverpa* was in range of (1.53 to 4.86 larvae/plant) in all the treated plots while (7.06 larvae/plant) recorded in untreated control.The treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha was proved to be superior and recorded least population of *Helicoverpa* (1.53 larvae/plant) and which was stand at par with emamectin benzoate 5 SG 220 gm/ha, lambda cyhalothrin 5 EC 250 ml/ha, indoxacarb 15.8 EC 250 ml/ha and spinosad 45 SC @ 125 ml/ha. The rest of the treatments fenvalerate 20 SC @ 400 ml/ha and profenophos 50 EC @ 1500 ml/ha were also significantly superior over control but proved second best.

Effect of newer insecticides on green boll damage after first spray

The data recorded before and after spraying are presented in Table-2. The green boll damage recorded one day before spraying was statistically non-significant. One dayafter first spray, the data recorded on one day after spraying shows that all the treatments recorded significantly lower (2.82 to 6.54% damage/plant) and control (6.82%/plant). The treatment of chlorantriniliprole 18.5 SC @ 150 ml/ha recorded least (2.82%/plant), which was significantly superior over rest of treatments and at par with emamectin benzoate 5 SG @ 220 gm/ha. The rest of all the treatments were secondly superior over control. Three days after first spray, the treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded least (1.67%/plant), and was found at par with emamectin benzoate 5 SG @ 220 gm/ha and lambda cyhalothrin 5 EC @ 250 ml/ha. The treatment of indoxacarb 15.8 EC @ 250 ml/ha and spinosad 45 SC @ 125 gm/ha were the second best treatments while, fenvalerate 20 EC @ 400 ml/ha and profenophos 50 EC @ 1500 ml/ha were exhibited poor performance and stand at par with control.

Seven days after first spray, the similar trend of superior treatment as three days after were observed here the treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded least green boll damage (3.29 per cent/plant) which was superior and at par with emamectin benzoate 5 SG @ 220 gm/ha and lambda cyhalothrin 5 EC @ 250 ml/ha. Next effective treatments were indoxacarb 15.8 EC @ 250 ml/ha spinosad 45 SC @ 125 gm/ha and profenophos 50 EC @ 1500 ml/ha, fenvalerate 20 EC @ 400 ml/ha was the poor treatment as statistically at par with control. Ten days after first spray, the observation recorded 10 days after spraying indicated that the treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded lowest green boll damage 1.57 per cent/plant and was stand at par with emamectin benzoate 5 SG @ 220 gm/ha, lambda cyhalothrin 5 EC @ 250 ml/ha and indoxacarb 15.8 EC @ 250 ml/ha while spinosad 45 SC @ 125 ml/hawas second best in damage reduction and rest of the treatment were statistically at par with control. Fourteen days after first spray, the data recorded on fourteen days after 1st spraying are presented in Table 2. The treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded least (1.65 per cent/plant) and which was at par with emamectin benzoate 5 SG @ 220 gm/ha whereas rest of all the treatments exhibited secondly superior performance except fenvalerate 20 EC @ 1500 ml/ha which was statistically at par with control.

Effect of newer insecticides on green boll damageafter second spray

The data on green boll damage before and after second spray (Table-2) were statistically non significant indicating uniform distribution of green boll damage one day before spraying. One day after second spray, the data pertaining to green boll damage one day before spraying shows that the treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded lowest damage (1.53%/plant), which was at par with emamectin benzoate 5 SG @ 220 gm/ha, lambda cyhalothrin 5 EC @ 250 ml/ha and indoxacarb 15.8 EC @ 250 ml/ha. While rest of the treatments were also shows the significant reduction in green boll damage over control.

Three days after second spray, the treatment of chlorantraniliprole 18.5 SC @ 150 ml/ha recorded maximum reduction in the green boll damage i.e. 1.78%/plant and which was at par with lambda cyhalothrin 5 EC @ 250 ml/ha and emamectin benzoate 5 SG @ 220 gm/ha. Indoxacarb 15.8 EC @ 250 ml/ha, spinosad 45 SC @ 125 ml/ha and fenvalerate 20 EC @ 400 ml/ha were proved to be superior secondly over control whereas the treatment of profenophos 50 EC @ 1500 ml/ha was at par with control. Seven days after second spray, the data recorded (Table-2) indicated that the trend pertaining to the performance of the different treatments was remain constant even seven days after spray. The treatment of profenophos 50 EC @ 1500 ml/ha was the poor treatment but significantly superior over control.

Ten days after second spray, the data related to green boll damage ten days after spraying reflects the treatment of emamectin benzoate 5 SG @ 220 gm/ha recorded least (3.40%/plant), which was significantly superior and followed by chlorantraniliprole 18.5 SC @ 150 ml/ha. Lambda cyhalothrin 5 EC @ 250 ml/ha, spinosad 45 SC @ 125 ml/ha and fenvalerate 20 EC @ 400 ml/ha recorded 3.69, 4.05, 4.69 and 5.10%/plant. Indoxacarb 15.8 EC @ 250 ml/ha and profenophos 50 EC @ 1500 ml/ha were also significantly superior over control. Fourteen days of second spray, the data recorded on fourteen day after spraying are presented in Table-2. All the treatments recorded significant reduction in per cent damage (4.69 to 6.67%/plant) against control (10.61%/plant).

Table-1: Population of *Helicoverpa* per plant after first and second spray

T. No	Treatments	Dose/ 10 lit	Dose/ ha	Population of *Helicoverpa*/plant before and after 1st spray							Population of *Helicoverpa*/plant before and after 2nd spray				
				Pre count	Pre count	1 DAS	3 DAS	7 DAS	10 DAS	14 DAS	1 DAS	3 DAS	7 DAS	10 DAS	14 DAS
1	Emamectin benzoate 5 SG	4 gm	220 gm/ha	5.68 (2.49	4.80 (2.31)	2.00 (1.59)	2.64 (1.79)	3.84 (2.08)	2.16 (1.63)	2.00 (1.59)	1.86 (1.54)	2.76 (1.81)	2.34 (1.53)	2.06 (1.60)	3.30 (1.95)
2	Lambda cyhalothrin 5 EC	5 ml	250 ml/ha	4.86 (2.32)	4.80 (2.31)	2.60 (1.76)	2.64 (1.79)	3.00 (1.87)	1.59 (1.42)	2.60 (1.76)	1.60 (1.45)	2.93 (1.85)	2.06 (1.60)	2.24 (1.65)	4.06 (2.14)
3	Chlorantraniliprole 18.5 SC	3 ml	150 ml/ha	4.80 (2.3)	6.70* (2.68)	1.59* (1.42)	2.16* (1.63)	2.46* (1.72)	2.00 (1.59)	1.53* (1.42)	1.53 (1.39)	1.53 (1.42)*	2.25 (1.50)	1.80 (1.52)	2.60 (1.76)*
4	Indoxacarb 15.8 EC	5 ml	250 ml/ha	5.33 (2.41)	5.73 (2.50)	3.00 (1.87)	3.33 (1.96)	2.00 (1.59)	1.53* (1.42)	3.00 (1.87)	2.13 (1.62)	3.30 (1.95)	2.22 (1.65)	2.36 (1.69)	4.66 (2.27)
5	Spinosad 45 SC	2.5 ml	125 ml/ha	6.13 (2.57)	4.00 (2.12)	3.01 (1.88)	4.02 (2.17)	2.60 (1.76)	1.59 (1.42)	3.02 (1.88)	2.43 (1.71)	3.33 (1.96)	2.85 (1.69)	1.75 (1.5)	5.67 (2.49)
6	Fenvalerate 20 EC	8 ml	400 ml/ha	6.53 (2.65)	4.53 (2.24)	3.56 (2.01)	4.68 (2.28)	2.00 (2.64)	2.00 (1.59)	3.56 (2.01)	2.53 (1.74)	3.73 (2.06)	1.66 (1.78)	2.66 (1.78)	2.93 (1.85)
7	Profenophos 50 EC	30 ml	1500 ml/ha	7.00 (2.74)	4.60 (2.66)	4.86 (2.32)	5.73 (2.50)	2.64 (1.79)	3.84 (2.08)	4.86 (2.32)	2.68 (1.79)	4.33 (2.20)	2.98 (1.86)	2.95 (1.86)	6.90 (2.72)
8	Untreated control	-	Water spray	6.90 (2.72)	6.20 (2.59)	6.30 (2.60)	9.54 (3.19)	5.73 (5.73)	6.20 (2.59)	7.06 (2.75)	5.67 (2.49)	5.50 (2.44)	4.46 (2.23)	7.00 (2.74)	8.24 (2.96)
	S.E.±			0.14	0.15	0.14	0.14	0.19	0.20	0.15	0.12	0.16	0.11	0.11	0.16
	C. D. at 5 %			NS	NS	0.43	0.43	0.59	0.62	0.45	0.36	0.50	0.34	0.33	0.50
	C.V. (%)			9.81	11.32	11.99	11.25	11.38	10.51	12.78	11.17	14.61	11.29	11.20	11.22

* Figures in parenthesis are transformed angular values.

Table-2: Effect of newer insecticides on green boll damage per plant on non *Bt* cotton after first and second spray

Tr. No	Treatments	Doses / 10lit	Doses/ha	Percent green boll damage / plant after 1st spray						Percent green boll damage / plant after 2nd spray					
				Pre count	1 DAS	3 DAS	7 DAS	10 DAS	14 DAS	Pre count	1 DAS	3 DAS	7 DAS	10 DAS	14 DAS
1.	Emamectin benzoate SG	4 gm	220 gm/ha	5.15 (13.10)	2.00 (8.1)	2.30 (8.72)	2.87 (9.68)	3.40* (10.52)	5.10 (13.05)	5.04 (13.10)	3.10 (10.1)	3.24 (8.44)	3.64 (10.97)	1.84 (7.87)	2.84 (9.66)
2.	Lambda cyhalothrin 5 EC	5 ml	250 ml/ha	5.46 (13.49)	2.35 (8.75)	2.78 (9.6)	3.42 (10.7)	4.05 (11.57)	4.69* (12.19)	4.94 (11.42)	4.04 (11.59)	2.85 (9.71)	3.96 (11.54)	2.35 (8.8)	3.92 (11.42)
3.	Chlorantraniliprole 18.5 SC	3 ml	150 ml/ha	4.38* (12.06)	1.53* (7.16)	1.78* (7.70)	2.23* (8.55)	3.69 (11.04)	5.64 (13.49)	5.56 (13.61)	2.82* (8.69)	1.67* (7.37)	3.29* (10.34)	1.57* (7.13)	1.65* (7.36)
4.	Indoxacarb 15.8 EC	5 ml	250 ml/ha	5.12 (13.05)	(2.87) (9.78)	3.40 (10.52)	4.15 (11.73)	5.45 (13.49)	5.40 (13.49)	5.10 (13.05)	4.84 (12.70)	3.47 (10.67)	4.38 (12.04)	2.77 (9.58)	5.10 (13.05)
5.	Spinosad 45 SC	2.5 ml	125 ml/ha	5.34 (13.33)	3.69 (11.04)	4.05 (11.57)	4.94 (12.83)	4.69 (12.19)	6.67 (15.16)	5.33 (13.33)	5.57 (13.63)	4.23 (11.84)	4.98 (12.86)	3.45 (10.71)	5.90 (14.07)
6.	Fenvalerate 20 EC	8 ml	400 ml/ha	6.66 (15.15)	4.69 (12.19)	4.56 (12.35)	5.74 (13.92)	5.10 (13.05)	5.16 (13.12)	6.85 (15.15)	6.54 (14.79)	5.01 (12.93)	5.68 (13.77)	4.58 (12.36)	6.82 (15.15)
7.	Profenophos 50 EC	30 ml	1500 ml/ha	4.70 (12.52)	5.46 (13.49)	5.45 (13.49)	6.53 (14.83)	6.60 (18.01)	5.46 (13.46)	6.65 (14.36)	5.05 (13.10)	5.92 (14.08)	4.84 (12.70)	5.74 (13.89)	4.70 (12.52)
8.	Untreated control	-	Water spray	3.94 (11.42)	4.84 (12.69)	5.32 (13.33)	8.26 (16.65)	9.55 (18.01)	10.61 (19.01)	6.54 (14.79)	7.35 (15.75)	5.90 (14.07)	8.20 (16.65)	6.65 (14.36)	7.35 (15.75)
	S.E.±			0.77	1.04	0.78	0.84	0.89	0.95	0.77	0.93	0.84	0.42	0.81	1.10
	C. D. at 5 %			NS	3.16	2.37	2.54	2.71	2.89	NS	2.82	2.54	1.27	2.45	3.34
	C.V. (%)			8.98	12.43	11.43	12.30	12.30	12.89	10.49	11.45	11.74	11.77	11.90	12.54

The treatment of lambda cyhalothrin 5 EC @ 250 ml/ha was emerged as a superior treatment and recorded lowest green boll damage i.e 4.69 per cent/plant. The rest of all the treatments were also at par with lambda cyhalothrin 5 EC @ 250 ml/ha except spinosad 45 SC @ 125 ml/ha which was next best treatment. Several workers have reported similar findings that application of emamectin benzoate, chlorantraniliprole, Lambda-cyhalothrin, spinosad and indoxacarb at recommended dose effectively reduced the damage of bollworm with increased grain yield than control (RAGHURAMAN *et al.,* 2008 and BRICKLE *et al.,*1999). The newer insecticides like emamectin benzoate, lambda cyhalothrin and profenophos proved to be most effective in reducing fruiting bodies damage and increasing seed cotton yield.The present findings are in conformity with the findings of RANGANATHAN and GOVINDAN (1996); BHEEMANNA *et al.* (2005); PAWAR and JADHAV (1989).

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INFLUENCE OF PROBIOTIC ON THE RELATIVE CONDITION FACTOR AND RELATIVE GROWTH CO-EFFICIENT OF *CARASSIUS AURATUS*

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ABSTRACT: The study was designed for a period of 90 days to evaluate the effect of probiotic Aqualact at different levels on relative condition factor and relative growth co-efficient of *Caraciuss auratus*. The results showed that the relative condition factor (Kn) and relative growth co-efficient (b) in fishes that fed with diets containing probiotic was significantly ($P<0.05$) higher than control group. Further, the species attained isometric growth and good condition fed with diets containing probiotic @ 0.6% indicating the significant influence of the probiotic.

Key words: *Carassius auratus*, probiotic, growth co-efficient, relative condition factor

INTRODUCTION

The goldfish (*Carassius auratus*), belongs to the family Cyprinidae of order Cypriniformes is one of the most commonly kept aquarium fishes and oldest cultured fish in the world. In India Aquarium hobby is nearly 70 years old and dates back to pre-independence era (AYAPPAN *et al.,* 2006). India has recorded at least 150 commercially important ornamental fish species and trade mainly indigenous freshwater species collected from rivers (MADHU *et al*., 2009). FAO has designated the use of probiotics as a major means for the improvement of aquatic environmental quality (SUBASINGHE *et al*., 2003).

Probiotic bacteria could produce digestive enzymes and essential growth nutrients such as vitamins and amino acids, which are benefit for enhancing the best growth, also they could benefit to their invertebrate host by competitive exclusion against pathogens (GOMEZ *et al*., 2000). The effects of probiotics on cultured aquatic animals have emphasized a reduction in mortality or conversely, increased survival and improved resistance against diseases (VILLAMIL *et al*., 2003). The relative growth coefficient and relative condition factor in length-weight relationship of fishes are considered as the important parameters for understanding of fish.

MATERIALS AND METHODS

The experimental goldfish juveniles were procured from seed production centre, Govt of Odisha. The fishes were allowed to acclimatize to the laboratory conditions for 7 days and then used for the experimental studies. The *Carassius auratus* were stocked at density of 10 nos per glass aquarium (2×1×1cubic feet). The fishes were divided randomly into six groups and each group was in three repetitions in separate aquarium. The untreated basal diet served as control (T_0) and the diet mixed with probiotic strains were considered as treatments (T_1, T_2, T_3, T_4 and T_5). The fishes were fed twice daily at the rate of 3% (average body weight) up to 90 days. The basal diet contained probiotic Aqualact @ 0.3%, 0.4%, 0.5%, 0.6% and 0.7% for the treatments T_1, T_2, T_3, T_4 and T_5, respectively. Water quality parameters were monitored throughout the experimental period. Required water quality was maintained by periodic partial exchange of water.

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Water samples collected at each sampling were analyzed for pH, temperature, dissolved oxygen, free carbon dioxide, NH3, and total alkalinity by following standard methods (APHA, 1985). The sampling was carried at an interval of 15 days to measure the length and weight of the species. The length-weight relationship is calculated using the following formula developed by LE CREN (1951).

$W=aL^b$ Where, W is the body weight, L is the total length, a and b are constants.
The relative condition factor (Kn) is calculated by Kn= Wo/Wc
Where, Wo is the observed weight and Wc is the calculated weight of the species

RESULTS AND DISCUSSION

The mathematical relationship between length and weight of fishes provides information on the changes in the well-being of the fishes that happens during the culture period. In the present study, the total length of the species varied from 3.16 cm to 8.14 cm, 3.10 cm to 8.40 cm, 3.18 cm to 8.30 cm, 3.09 cm to 8.70 cm, 3.03 cm to 8.83 cm and 3.16 cm to 8.40 cm in T_0, T_1, T_2, T_3, T_4 and T_5 respectively. The body weight varied from 0.60 g to 5.69 g, 0.63 g to7.20 g, 0.60 g to 7.80 g, 0.63 g to7.20 g, 0.66 g to 9.02 g and 0.63 g to 7.20 g respectively for T_0, T_1, T_2, T_3, T_4 and T_5 over a experimental period of 90 days. In this research there was no significant differences ($P>0.05$) in initial weight between the control and treatments. Similarly no significant difference ($P>0.05$) was found in initial length between control and treatments of the species during the stocking.

The length-weight relationship of *Carassius auratus* in T_0 is derived as
$W =0.043L^{2.257}$ (Fig.1)
and its logarithmic transformation is calculated as LogW= -1.3665 +2.257LogL
For T_1, $W= 0.041L^{2.302}$ (Fig.2)
and its logarithmic transformation is calculated as Log W= -1.3872 +2.302LogL
For T_2, $W= 0.039L^{2.320}$ (Fig.3)
and its logarithmic transformation is calculated as Log W= -1.4089+2.320LogL
For T_3, $W=0.037L^{2.357}$ (Fig.4)
and its logarithmic transformation is calculated as Log W= -1.4318+ 2.357LogL
For T_4, $W=0.033L^{2.827}$ (Fig.5)
and its logarithmic transformation is calculated as Log W= -1.4814+ 2.827LogL
For T_5, $W= 0.040L^{2.307}$ (Fig.6)
and its logarithmic transformation is calculated as Log W=-1.3979 + 2.307LogL

The “b” value in the length-weight relationship of *Carassius auratus* was found to be 2.257, 2.302, 2.320, 2.357, 2.827 and 2.307, respectively for T_0, T_1, T_2, T_3, T_4 and T_5. The higher “b” value was obtained in T_4 (basal diet containing probiotic Aqualact @0.6%). The numerical value of “b” in the length-weight relationships of *Carassius auratus was* within the acceptable and expected range of 2.5 and 3.5 (FROESE, 2006). However, the remaining control and treatments showed “b” values below 2.5 ($b<2.5$). PATRA *et al.* (2000) stated that the departure of “b” values from 3.0 would be due to the seasonal changes while, LE CREN (1951) opined that the deviation of “b” value from isometric growth either due to environmental condition or condition of fish. If “b” value is different from 3, weight growth is said to be allometric (fish changes shape as it grows larger).

In fishes, generally the growth pattern follows the cube law (BRODY, 1945; LAGLER, 1952). The “b” value in the treated aquarium (basal diet containing probiotic Aqualact @0.6%) was very closer to the value of isometric growth ($b=3.0$). The results of statistical analysis showed that the value of exponent (b) of the length-weight relationships was not significantly different from the cube value (3). The value being

closer to 3 indicated an isometric growth. This could be due to the higher weight gain resulting by the positive impact of probiotic. The "b" value was lower in the control (T_0), while higher in T_4. There is a clear indication of the positive effect of probiotic on "b" values up to the basal diet containing probiotic Aqualact @0.6% and the "b" value declined, when the dose increased to 0.7% (T_5),.

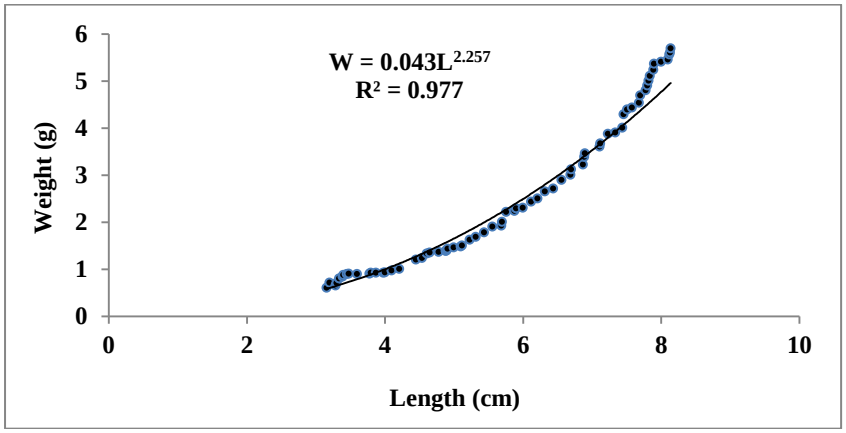

Fig.1: Length-weight relationship of *Carassius auratus* in Control (T_0)

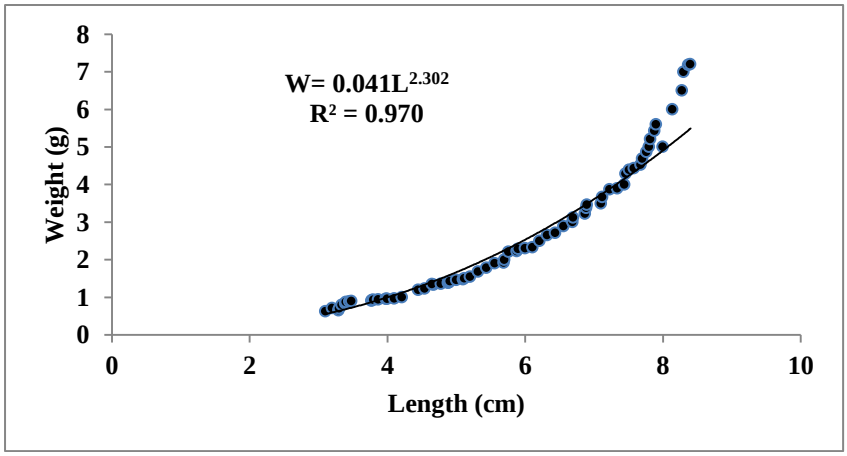

Fig.2: Length-weight relationship of *Carassius auratus* in T_1

Further, the relative condition factor (Kn) was calculated as 0.76, 0.79, 0.79, 0.84, 0.93, 0.78 for T_0, T_1, T_2, T_3, T_4 and T_5 respectively. The higher value (0.93) was obtained at T_4 (diet containing probiotic Aqualact @0.6%). LE CREN (1951) proposed the relative condition factor in preference to condition factor as the latter is influenced by many environmental and biological factors. Condition factor measures the deviation from a hypothetical ideal fish where as relative condition factor measures the deviation from the average weight or length of fish. GEORGE *et al*. (1985) reported that Kn indicates the general well-being of the fish. If the values of Kn greater than one (1) indicates that the well-being of the fish is good whereas, its value less than 1 reflects that the well-being of the fish is not in a good condition.

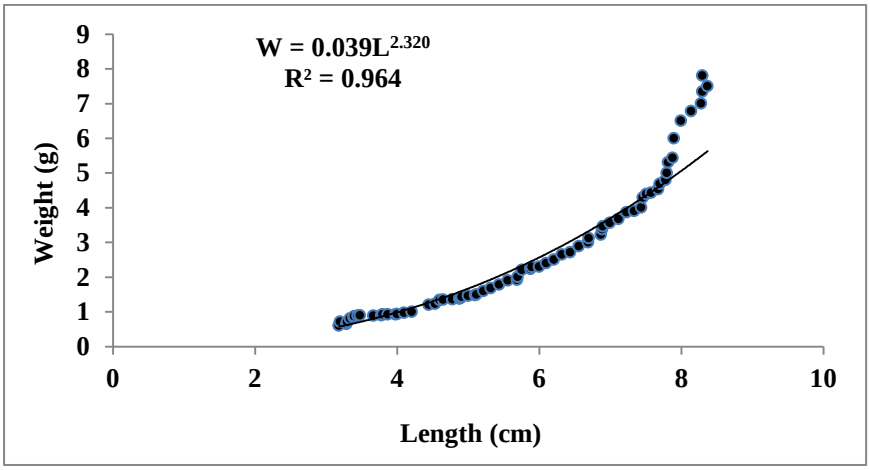

Fig.3: Length-weight relationship of *Carassius auratus* in T_2

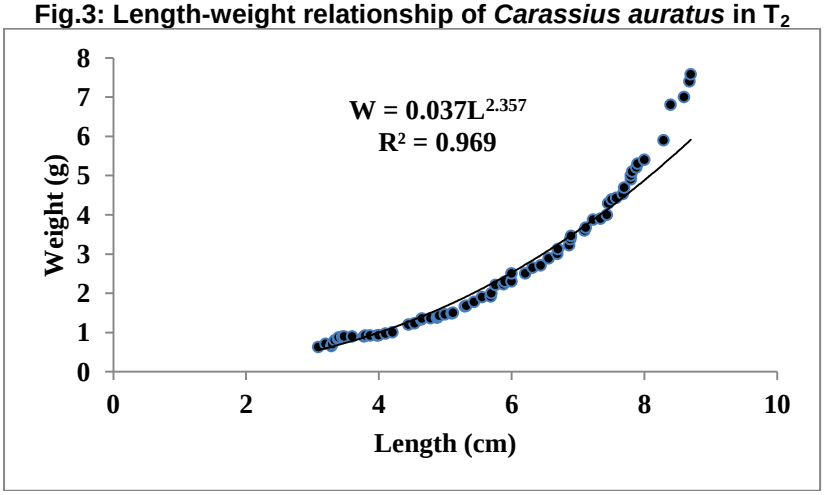

Fig.4: Length-weight relationship of *Carassius auratus* in T_3

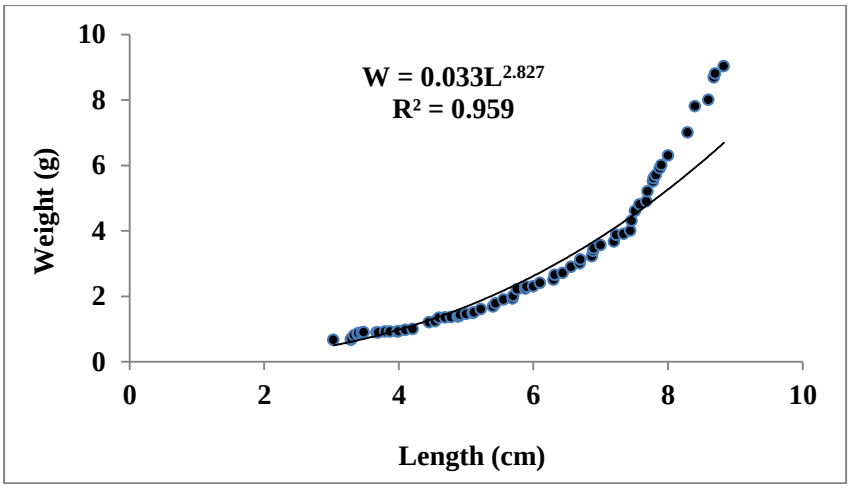

Fig.5: Length-weight relationship of *Carassius auratus* in T_4

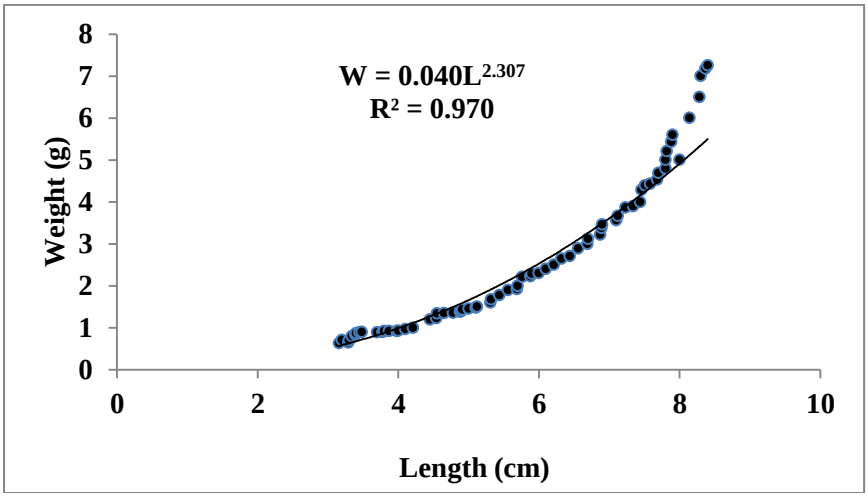

Fig.6: Length-weight relationship of *Carassius auratus* in T_5

Higher value of Kn may be due to several reasons such as feeding intensity, maturity, breeding cycle or environmental factor (SHAFI and QUDDUS, 1974; MANORAMA and RAMANUJAM, 2014) and the decline in Kn values could have been caused by the dominance of larger specimens in the samples and also by the spent gonads (MASUD and SINGH, 2011; MANORAMA and RAMANUJAM, 2014). Usually Kn values nearing 1.0 or more than 1.0 are considered as good condition of index reported by KUND *et al.* (2011) confirms the present study indicating a negligible variation between observed weight and estimated weight of *Carassius auratus* cultured in the aquaruim with basal diet containing probiotic Aqualact @0.6%.

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COMMENSAL ASSOCIATION IN PISCIVOROUS BIRDS

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ABSTRACT: Studies on habitat use and foraging ecology of piscivorous birds were made on Nyari-I reservoir from September 1998 to October 1999. Foraging association between mass foraging cormorants and terns was recorded. The association was widespread in other places as well. We could also observe Dalmatian Pelican, Intermediate Egret, Little Egret associating with mass foraging cormorants. We present indices of association to show the significance of association and also brief the significance changes in the behavior of species joining mass foraging cormorants.

Key Words: Commensal association, terns, cormorants, pelicans, egrets, piscivorous birds

INTRODUCTION

Commensal associations have been documented in piscivorous birds. Many of the observations reveal herons and egrets associate with other birds (MEYERRIECKS, 1967; EMLEN and AMBROSE, 1970; MACDONALD, 1981; RIBIC *et al*., 1997; BENNETT SMITHSON, 2001; KYLE, 2005; PARASHARYA and MATHEW 1994). RIDOUX (1987) observed *Orcinus orca* pods and associated Coastal bird flocks, Giant Petrels, *Macronectes* sp., Cape Pigeon, *Daption capense*, and Kelp Gull, *Larus dom* from the Coast of Possession Island. HODGES and WOEHLER 1994 recorded associations between seabirds and cetaceans. ORO (1995) made two observations of Audouin's Gull flocks foraging on small fish forced to rise to the sea surface by tunas. BRAGER (1998) investigated the relationship between White-fronted Terns (*Sterna striata*) associating with Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand. BALASUBRAMANIAN (1990) reported feeding association between Jackal, *Canis aureus* (Linnaeus) and two species of egrets in Tamil Nadu, India. CAMPHUYSEN and WEBB (1999) studied the foraging distribution and the formation of multi-species feeding associations of seabirds and marine mammals of the British east coast. Present study is based on our observations on Nyari-I reservoir from September 1998 to October 1999. We could observe formation of group foraging and association of cormorants with terns, herons and egrets and pelicans. There is little information on consequences/ effects of commensal associations on the behavioral changes and energy gain or loss of the birds.

MATERIALS AND METHODS

The study area is located at 20º10'N to 24º45'N latitude and 68º10'E to 72º30'E longitudes in the Gujarat state, India (Fig. 1). The soils are poor, with half decomposed rocks called "murrum". Sand, sandstone and black cotton soils are the major soil types (WYNTER-BLYTH 1962). The climate is dry tropical monsoon type. Three distinct seasons are experienced i.e. winter, summer and monsoon. Relative humidity ranges

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from 66.0 to 82.0% and average rainfall is 620 mm (Table-1). The area has many dykes, suitable for impoundments which encouraged people of the region to make irrigation dams and village ponds. GARG *et al.* (1998) described 352 inland and coastal wetlands covering 259800.00 ha in Saurashtra which includes 209206.00 ha of inland wetlands. LATHIGARA (2001) listed 185 irrigation dams in Saurashtra. Water spread area of many wetlands is undulating and many hillocks are present which creates temporary / permanent islands in the dam. The constant changes in the water level create various temporary habitats for birds. These changes in water level affects wetland birds either positively or negatively depending upon their feeding niche or feeding habits.

Extensive observations were made at Nyari-I reservoir near Rajkot city. The reservoir is 18 m high at dam wall. Base of the water spread area is undulating. Hillocks emerge as temporary or permanent islands in the reservoir. When water surface reaches two meters at 'dam wall' reservoir divides into two puddles. One which recede in dam wall ('Dam wall' site) and the other confines to Pumping Station ('Pumping Station' site) built for the water supply to Rajkot city.

RESULTS AND DISCUSSION

Commensal association was mainly observed when mass foraging groups of cormorants were observed. Cormorant group activity was observed in winter and summer season in present study. The study was carried out from September 1998 to October 1999. Thus the study coincided with two winter and one summer season. Mass fishing in *Phalacrocorax carbo sinensis* has been new phenomenon and is an adaptation to turbid environment (VAN EERDEN *et al.* 1995). Our observations indicated instances of solitary foraging activity in Little Cormorant and Large Cormorant even when mass foraging activity was being observed indicating strong kinship forces governing mass foraging activity.

Three species of Phalacrocoracidae *viz.* Little Cormorant, Great Cormorant and Indian Shag are observed in the region. Mixed species flock of cormorants forage in a typical group of individuals which may be few individuals to 100 or more individuals. A flock foraged for 15-30 minutes on an average. Mass foraging group of cormorants periodically arrived ashore and was observed wing spreading. Wing spreading behavior is observed exclusively in cormorants; several reasons have been proposed for this behavior (HENNEMANN 1982, 1984). The most appropriate explanation for their wing spreading behavior is drying the wing feathers.

River Tern is common in the region. River Tern prefers to forage solitarily. Thus individuals of River Tern may be found flying here and there within the irrigation dam. A small group of nine or ten individuals or a large group as large as 100 or more individuals can be found resting ashore during day time. Arrival at resting place or leaving for foraging of an individual was never observed in groups/flocks. Time to start foraging activity and the time at which the bird go to roosting site varied for cormorants and terns. Cormorants arrived at foraging site after half an hour of sunshine time and departed to roosting half an hour before sunset time. River Tern started their activity half an hour before sunshine time and continued till half an hour later sunset time. Terns had established roosting sites on the islands in the Nyari-I reservoir only. River Tern arrived at night roosting site in large flocks. Counting of River Tern was difficult on the site even though River Tern roost at night in large number at this reservoir due to very low visibility of dusk. We frequently observed association between cormorant and tern. Little Egret and Intermediate Egret, also joined in such association while foraging cormorants arrived ashore. In the absence of cormorants, tern foraged by their natural flocking as well as

solitary method. Dalmatian Pelican was also observed to participate in the group foraging during second winter season (September- October 1999).

Table-1: Number of times Little Cormorant, Large Cormorant and River Tern observed in Solitary and Group foraging activity

		Little Cormorant		River Tern		Large Cormorant	
		Solitary	**Group**	**Solitary**	**Group**	**Solitary**	**Group**
Post Monsoon-I	<4.5 m	37	1	32		10	
Post Monsoon-I	4.5 m - 2 m	15	2	6		3	
Winter-I	<4.5 m	16	30	13	14	16	3
Winter-I	4.5 m - 2 m	1	6	3	6		
Summer-I	4.5 m - 2 m	1	19		19		
Monsoon-I	2 m - 0.5 m		1	1	2	2	4
Monsoon-I	>0.5 m	10	5	16	27		
Winter-II	>0.5 m	1	1	1	1	1	1
Summer-II	>0.5 m	28	1	4		8	
Monsoon-II	>0.5 m	3	3	7	3		1

Number of times Little Cormorant, Large Cormorant and Indian River Tern observed in solitary and group foraging activity recorded in Table-1 revealed that solitary foraging was more prevalent in Indian River Tern. Indian River Tern showed group foraging activity when cormorant species were engaged in group foraging. Large Cormorant was infrequent and was observed foraging with Little Cormorant except in Monsoon-II when large number of wading birds was engaged in group foraging activity and Indian River Tern was also observed in these groups. Cormorants were absent in these groups.

Indices of association

An attempt has been made to calculate association between Cormorant and Tern. Solitary foraging activity has not been considered in the analysis as solitary foraging do not attract feeding association. Both the cormorant species are considered together because both the species engaged in group foraging activities together. Following formula was used.

In present study

$N_{AB\ (CORMORANT+TERN)} = 65$
$N_{A\ (CORMORANT)} = 8$ and
$N_{B\ (TERN)} = 23$
Index of association was 0.68, which shows that the association was real.

Table-2 shows mean and Standard Deviation for Little Cormorant, Large Cormorant and River Tern. Table-1 clearly shows that most of the group formation and group activities had been observed in Margin area. Margin is the peripheral area of the irrigation dam where water level is varying from shallow to little deep. Damwall and Pumping Station sites are deep sites divided by rocky mound. The birds did not forage in this area until drying of the dam. The Margin area dried up in later part of the study and could not serve for the foraging of the birds (<2 m; Table-2).

Behavioral changes observed due to association

Change in diurnal activity rhythm

River Tern foraged solitarily in normal conditions. Its activity started early in the morning, half an hour before sunshine. After 9:00 am the birds rested on an undisturbed resting site ashore of the irrigation dam. The birds partially were observed foraging during this time. The birds became active in the evening at 4:30-5:00 pm again.

Fig. 2: Mass foraging Little Cormorants and Great Cormorants with associated Dalmatian Pelican and River Tern at Nyari-I reservoir

The birds continued foraging up to late in the evening and went to roost half an hour after sunset. River Tern foraged in loose small or large group when mass foraging cormorants were present. They synchronized their foraging time with cormorants i.e. it was observed foraging while cormorants started foraging and went to the resting site when cormorants went for resting a typical behavior of cormorants during which cormorants do wing spreading. Terns did not follow their diurnal activity rhythm.

However, terns did not change their total activity time. In given situation, terns foraged naturally in the morning till cormorants arrived from the roosting. The time at which terns started their activity and the time cormorants arrived to forage had approximately an hour's difference. In the evening when cormorants left for roosting they again foraged their normal way for approximately an hour till it went to roost. Dalmatian Pelican normally forages in group. They make a circle, draw fishes within the circle and then make feeding attempts (ALI, 1996). When mass foraging cormorants were present Rosy Pelicans joined foraging cormorants and followed cormorants rather than forming circle. Pelicans too preferred to rest when cormorants went to the resting site and followed them when cormorants went foraging. Their resting site was different from cormorants and was far away from where cormorants could rest. We have observed

complete change in diurnal activity rhythm of River Tern and Dalmatian Pelican. Figure 1 show mass foraging cormorants with River Tern, Dalmatian Pelican and Egret.

Table-2: Descriptive statistics of Large Cormorant, Little Cormorant and River Tern in various parts of Nyari-I reservoir

Water Level	Species	Site in Nyari-I reservoir	Mean	SD
> 4.5 m	Large Cormorant	Pumping Station	1.08	0.72
> 4.5 m	Large Cormorant	Damwall	0.80	0.84
> 4.5 m	Large Cormorant	Margin	0.26	0.58
> 4.5 m	Little Cormorant	Pumping Station	30.72	59.48
> 4.5 m	Little Cormorant	Damwall	9.20	11.30
> 4.5 m	Little Cormorant	Margin	135.34	167.36
> 4.5 m	River Tern	Pumping Station	10.36	15.06
> 4.5 m	River Tern	Damwall	3.00	3.32
> 4.5 m	River Tern	Margin	14.04	13.27
4.5 m - 2 m	Large Cormorant	Pumping Station	0.00	0.00
4.5 m - 2 m	Large Cormorant	Damwall	0.00	0.00
4.5 m - 2 m	Large Cormorant	Margin	0.00	0.00
4.5 m - 2 m	Little Cormorant	Pumping Station	73.00	83.74
4.5 m - 2 m	Little Cormorant	Damwall	46.90	72.72
4.5 m - 2 m	Little Cormorant	Margin	182.50	252.17
4.5 m - 2 m	River Tern	Pumping Station	5.40	9.99
4.5 m - 2 m	River Tern	Damwall	27.20	28.49
4.5 m - 2 m	River Tern	Margin	53.00	68.80
2 m -0.5 m	Large Cormorant	Pumping Station	0.00	0.00
2 m -0.5 m	Large Cormorant	Damwall	0.50	0.71
2 m -0.5 m	Large Cormorant	Margin	0.00	0.00
2 m -0.5 m	Little Cormorant	Pumping Station	0.00	0.00
2 m -0.5 m	Little Cormorant	Damwall	1.15	1.63
2 m -0.5 m	Little Cormorant	Margin	0.00	0.00
2 m -0.5 m	River Tern	Pumping Station	5.50	7.78
2 m -0.5 m	River Tern	Damwall	5.80	3.54
2 m -0.5 m	River Tern	Margin	0.00	0.00
< 0.5 m	Large Cormorant	Pumping Station	3.50	6.06
< 0.5 m	Large Cormorant	Damwall	0.67	1.15
< 0.5 m	Large Cormorant	Margin	0.00	0.00
< 0.5 m	Little Cormorant	Pumping Station	13.28	23.00
< 0.5 m	Little Cormorant	Damwall	0.67	1.15
< 0.5 m	Little Cormorant	Margin	0.00	0.00
< 0.5 m	River Tern	Pumping Station	101.00	92.68
< 0.5 m	River Tern	Damwall	9.00	15.59
< 0.5 m	River Tern	Margin	0.00	0.00

SD = Standard Deviation

Change in behavior

Herons and Egrets forage by various feeding tactics. When we observed mass foraging cormorants foraging near shore herons and egrets synchronized their activity with foraging cormorants, they showed continuous changes in feeding tactics according to requirement to synchronize activity with foraging cormorants. If required they flew a few

meter and again joined with cormorants. However, they continued their foraging activity when group of cormorants went to the resting site and foraged by their normal way.

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BIO-EFFICACY OF BIO-RATIONAL INSECTICIDES AGAINST PINK STEM BORER, *SESAMIA INFERENS* WALKER OF MAIZE

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ABSTRACT: Study on the bio- efficacy of different bio-rational insecticides against pink stem borer under field conditions was conducted during Kharif 2016-17 at the Research cum Instructional Farm of Indira Gandhi Krishi Vishwavidyalaya,Raipur(C.G.).The plots treated with emamectin benzoate 5% SG depicted minimum number of pin holes (1.00) which was at par with spinosad 45% SC @ 125 ml ./ha (1.31), chlorantraniliprole 18.5% SC @ 150 ml /ha (1.56), and *Bacillus thuringiensis* @ 250 ml /ha (1.72).However, application of karanj oil @ 10 litre (2.32) was least effective but significantly superior over untreated control (3.55) per plant. The highest grain yield was recorded in emamectin benzoate 5% SG (40.97 q ha^{-1}). Application of emamectin benzoate, spinosad, chlorantraniliprole and *Bacillus thuringiensis* proved to be the best regarding grain yield of maize. The highest cost-benefit ratio was recorded with emamectin benzoate (7.91) while the lowest benefit: cost ratio was with karanj oil (1.63).

Key words: Bio-efficacy, bio-rational, maize, pink stem borer

INTRODUCTION

Maize (*Zea mays* L.) is the most completely domesticated crop among the cereals. Mexico or Central America is most likely being the origin of corn (MARTIN *et al*., 2002). It is one of the most important cereal crops next to wheat and rice in terms of total production in the world, India ranks fourth in area and fifth in production in the world. Around 250 species of insect and mite species attack maize in field and storage conditions (MATHUR, 1991). However, only about a dozen of these are quite serious (SIDDIQUI and MARWAHA, 1993). Among the different insect pests, stem borer species associated with maize in India are *Chilo partellus* Swinhoe and *Sesamia inferens* Walker, commonly known as pink stem borer. Losses due to *S. inferens* which is a major pest during post rainy season in south India varied from 25.7 to 78.9 per cent.

The pink stem borer, *Sesamia inferens* is one of the major insect pests of maize, that causes wide damage to the crop in peninsular India during rabi season. Foliage damage, stem tunneling, dead-heart,stem breakage, plant lodging, ear damage, and tassel damage are the various damages caused by this pest. Foliage damage, stem tunneling and dead heart are, however, the major ones that cause severe yield loss in maize (CHATTERJI *et al*., 1969, ATTRI *et al*., 1968, MATHUR *et al*., 1981). In India, it is reported as a pest in Andhra Pradesh, Karnataka, Tamilnadu, Madhya Pradesh, Maharashtra, Odisha, West Bengal, Bihar, Assam, Uttar Pradesh, Delhi and Punjab. (REDDY *et al.,* 2003).Injudicious and indiscriminate use of chemical pesticides in the past has created a number of problems like insecticide resistance, insecticide residues, pest resurgence, environmental pollution and direct and indirect hazards to human beings etc. Bio-rational strategies employing insect growth regulators, natural products, botanical preparations and entomopathogenic microbials are getting significance as possible alternative measures for the sustainable management of insect pests.

MATERIALS AND METHODS

The field experiment was carried out during Kharif 2016 using randomized block design with three replications and nine treatments along with an untreated check at the Research cum Instructional Farm of Indira Gandhi Krishi Vishwavidyalaya, Raipur(C.G.). The popular maize hybrid Sugar-75 was sown over a plot size 4x3 m for each treatment with spacing of 75x20 cm and the crop was raised by following recommended package and practices except plant protection measures. An experiment was conducted with eight bio-rational insecticides *viz*., indoxacarb 14.5% SC @ 500 ml /ha, azadirachtin 0.15% SC @ 1000 ml /ha, emamectin benzoate 5% SG @ 10 g/ha, *Bacillus thuringiensis* @ 250 ml/ha, chlorantraniliprole 18.5% SC @ 150 ml/ha, spinosad 45% SC @ 125 ml/ha, *Beauveria bassiana* 1.15 % WP @ 750 g/ha and karanj oil @ 10 litre.

Insecticides were applied twice on 15 and 30 days after emergence of crop. Pre treatment observation was recorded on one day before application of insecticide and post treatment observation was taken in 7 days and 15 days after application of insecticides. Number of pin holes / plant, tunnel length /10 plants and yield /plot was recorded in the experimental plots. Yield data was analyzed and yield differences among different treatments were calculated. While comparing the yield from different treatments, the per cent increase in yield over control was calculated using the following formula.

$$\text{Increase in yield over control (\%)} = \frac{(T - C)}{C} \times 100$$

Where, T = Yield from treated plot.
C = Yield from control plot.

The leaf injury infestation was subjected to square root transformation (These transformed values were analyzed statistically by using the techniques of analysis of variance for randomized block design and significance was tested by “F” test (COCHRAN and COX, 1957). Eight different insecticidal treatments were evaluated including untreated control for the assessment of their comparative performance against pink stem borer of maize.

Economics of different insecticides were worked out as per the market price of the commodities and wages prevailing during the course of studies. For economic analysis, the factors considered were cost of different insecticides and additional cost involved. Gross and net returns and benefit cost ratio were worked out. Value of increased yield over untreated control was calculated by multiplying the increased yield over control by prevailing market price of maize (Rs 1365 per quintal). The net profit over untreated control was worked out by deducting cost of insecticides and labour charges from price of increased yield over control. The benefit: cost ratio was also calculated using following formula:

$$\text{Benefit cost ratio} = \frac{\text{Net returns}}{\text{Total cost (Insecticides + labour charges)}}$$

RESULTS AND DISCUSSION

Data in respect of pre-treatment and post treatment observations on leaf injury rating by *S. inferens* after first application are presented in Table 1 and Fig. 1. Mean of the number of pin holes in the pre treatment observation ranged non significant among themselves. However, number of pin holes from 1.43 to 2.63 was observed on randomly

selected plants. It is clear that in post treatment observation after 7th days of treatment, there were significant differences among all the treatment with respect to the number of pin holes. All the treatment was significantly superior over untreated control. Among the treatments, emamectin benzoate 5% SG @ 10 g./ha was recorded the effective treatment with the minimum number of pin holes (1.02) followed by spinosad 45% SC @ 125 ml /ha (1.46), chlorantraniliprole 18.5% SC @ 150 ml /ha (1.56), *Bacillus thuringiensis* @ 250 ml /ha (1.63), azadirachtin 0.15% SC @ 1000 ml /ha (1.93), *Beauveria bassiana* 1.15 % WP @ 750 g./ha (2.03) and indoxacarb 14.5% SC @ 500 ml /ha (2.06) per plant , respectively. However, application of karanj oil @ 10 litre was least effective (2.43) but significantly superior over untreated control (2.80). Post treatment observation on the pin holes at 15th days of first application of insecticide, showed that all the treatments were significantly reduce the number of pin holes over control. The plots treated with emamectin benzoate 5% SG depicted minimum number of pin holes (1.40) which was at par with spinosad 45% SC @ 125 ml /ha (1.60) chlorantraniliprole 18.5% SC @ 150 ml /ha (1.63), *Bacillus thuringiensis* @ 250 ml/ha (1.70), azadirachtin 0.15% SC @ 1000 ml /ha (1.76) and *Beauveria bassiana* 1.15 % WP @ 750 g/ha (1.96) followed by indoxacarb 14.5% SC @ 500 ml /ha (2.00) and karanj oil @ 10 litre (2.26) was least effective but significantly superior over untreated control (3.36) per plant.

Table-1: Effect of insecticides on pin holes caused by *S. inferens* on maize crop

Treatments	**Pre Treat-ment**	**Observtion of First Spray**		**Mean**	**Pre Treat-ment**	**Observtion of Second Spray**		**Mean**
		7 DAS	**15 DAS**			**7 DAS**	**15 DAS**	
Indoxacarb 14.5%SC	1.57 (1.55)*	2.06 (1.75)	2.00 (1.42)	2.03	1.81 (1.63)	2.11	2.26 (1.78)	2.11
Azadirachtin 0.15% SC	1.5 (1.55)	1.93 (1.69)	1.76 (1.66)	1.84	2.16 (1.77)	1.85	2.07 (1.74)	1.85
Emamectin Benzoate 5% SG	1.1 (1.43)	1.02 (1.44)	1.40 (1.52)	1.21	1.60 (1.60)	0.79	1.16 (1.44)	0.79
Bacillus thuringiensis	0.67 (1.28)	1.63 (1.60)	1.73 (1.64)	1.68	2.63 (1.89)	1.76	1.90 (1.69)	1.76
Chlorantranili prole 18.5% SC	1.5 (1.56)	1.56 (1.60)	1.63 (1.59)	1.59	1.96 (1.69)	1.54	1.83 (1.67)	1.54
Spinosad 45 % SC	2.07 (1.65)	1.46 (1.56)	1.60 (1.59)	1.53	1.63 (1.60)	1.10	1.20 (1.47)	1.10
Beauveria bassiana 1.15% WP	1.37 (1.53)	2.03 (1.77)	1.96 (1.69)	1.99	2.03 (1.73)	2.03	2.13 (1.76)	2.03
Karanj oil	0.6 (1.25)	2.43 (1.85)	2.26 (1.80)	2.34	1.66 (1.62)	2.30	2.50 (1.86)	2.30
Control	2.67 (1.89)	2.80 (1.94)	3.36 (2.08)	3.08	3.66 (1.72)	4.03	4.20 (2.27)	4.03
SEm(±)	**NS**	**0.09**	**0.16**		**NS**	**0.13**	**0.14**	
CD at 5 %		**0.25**	**0.38**			**0.41**	**0.43**	

*Figures in parentheses are square root transformed values; DAS = Days after spray

Number of pin holes caused by *S. inferens* after 2nd application of the insecticides
Data in respect of pre-treatment and post treatment observations on number of pin holes caused by *S. inferens* after second application are presented in Tables and Figs. The observations after 7th days of second application, all the tested doses of insecticides exhibited significant differences over control. Among the treatments with emamectin benzoate 5% SG recorded as the best effective treatment with minimum number of pin holes (0.43) per plant which was followed by spinosad 45% SC @ 125 ml./ha (1.00), chlorantraniliprole 18.5% SC @ 150 ml/ha (1.26), *Bacillus thuringiensis* @ 250 ml /ha (1.63), azadirachtin 0.15% SC @ 1000 ml /ha (1.63), *Beauveria bassiana* 1.15 % WP @ 750 g/ha (1.93) and indoxacarb 14.5% SC @ 500 ml /ha (1.96) per plant , respectively. However, application of karanj oil @ 10 litre (2.10) was least effective but significantly superior over untreated control (3.86) per plant. Post treatment observation on the pin holes at 15th days of second application of insecticide, showed that all the treatments were significantly reduce the number of pin holes over control. The plots treated with emamectin benzoate 5% SG depicted minimum number of pin holes (1.16) which was at par with spinosad 45% SC @ 125 ml//ha (1.20), chlorantraniliprole 18.5% SC @ 150 ml./ha (1.83), and *Bacillus thuringiensis* @.250 ml /ha (1.90) followed by azadirachtin 0.15% SC @ 1000 ml /ha (2.07), *Beauveria bassiana* 1.15 % WP @ 750 g/ha (2.13) and indoxacarb 14.5% SC @ 500 ml /ha (2.26), respectively. However, application of karanj oil @ 10 lt (2.50) was least effective but significantly superior over untreated control (4.20) per plant.

Table-2: Economics of different insecticides used for the management *S. inferens*

Insecticides	**Dose /ha**	**Yield q/ha**	**Increased yield over control (q/ha)**	**Price of yield increased over control (Rs./ha)**	**Net profit over control**	**Benefit : cost ratio**
Indoxacarb 14.5% SC	500 ml	27.18	9.67	13199.55	7407.55	1.98
Azadirachtin 0.15%SC	1000ml	30.96	13.45	18359.25	15167.25	6.03
Emamectin Benzoate 5% SG	10g	40.97	23.46	32022.90	28430.90	7.91
Bacillus thuringiensis	250ml	31.55	14.04	19164.60	16472.60	6.11
Chlorantraniliprole 18.5% SC	150ml	32.14	14.63	19969.95	12387.95	1.63
Spinosad 45% SC	125ml	34.33	16.82	22959.30	15274.80	2.98
Beauveria bassiana 1.15 % WP	750g	28.18	10.67	14564.55	11842.55	4.35
Karanj oil	10litre	26.37	8.86	12093.90	7501	1.63
Untreated control	-	17.51	0	0	0	0

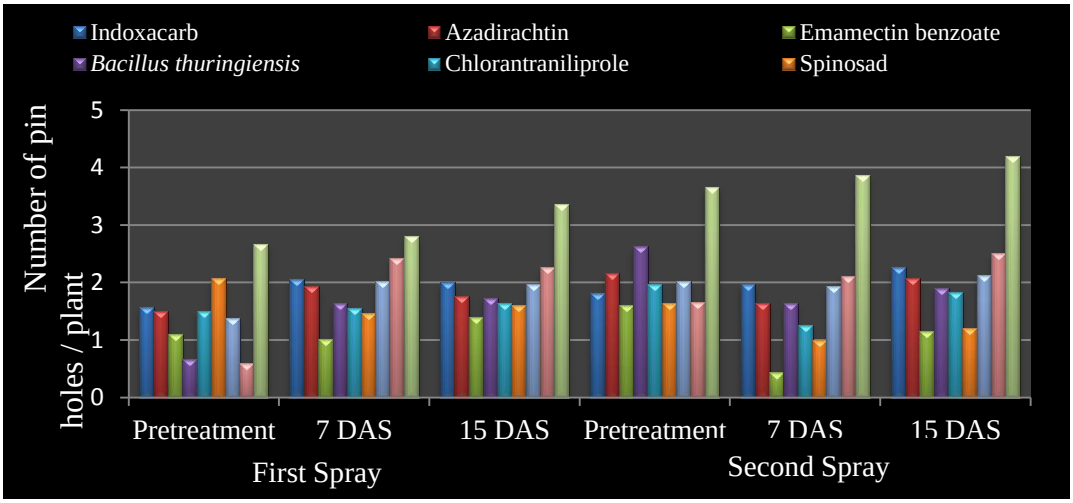

Fig-1: Effect of insecticides on pin holes caused by pink stem borer, *S. inferens* on maize crop

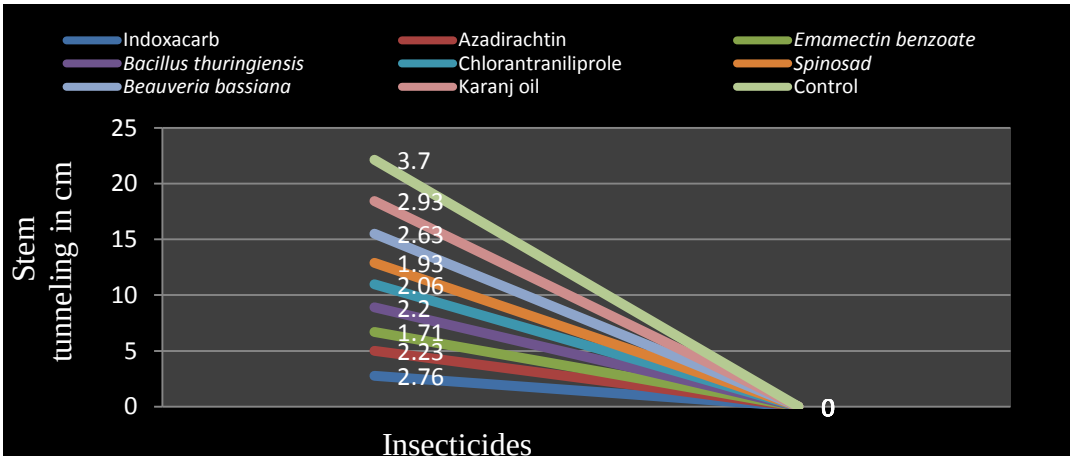

Fig-2: Effect of insecticides on stem tunnel length (cm) caused by *S inferens* on maize crop

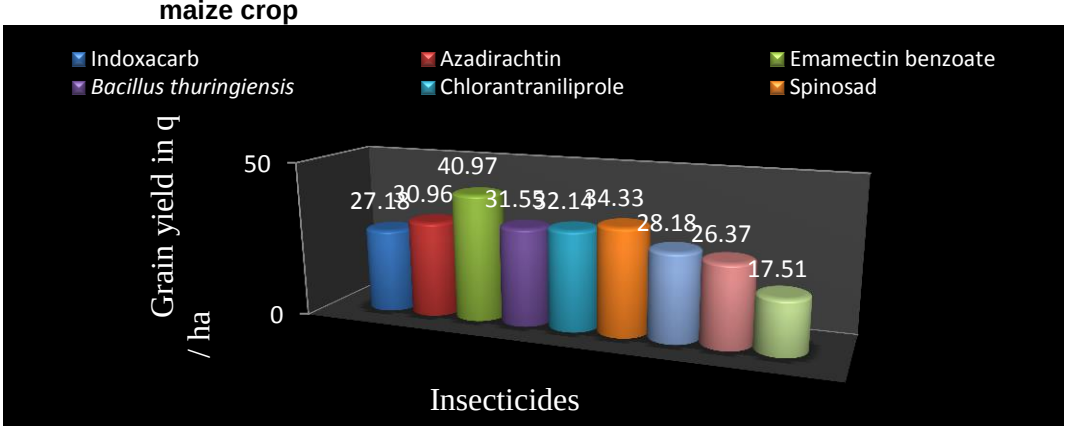

Fig-3: Showing grain yield of different insecticides treated plots

Effect of insecticides on stem tunnel length (cm)

On maize, stem tunnel length was formed due to the damage of *Sesamia inferens* by feeding inside the stem. The lowest stem tunnel length was recorded in Emamectin benzoate 5% SG (1.71 cm) which was followed by spinosad 45% SC (1.93 cm), chlorantraniliprole 18.5% SC (2.06 cm), *Bacillus thuringiensis* (2.2 cm), azadirachtin 0.15% SC (2.23 cm), *Beauveria bassiana* 1.15 % WP (2.63 cm) and indoxacarb 14.5% SC (2.76 cm), respectively, while the highest stem tunnel 2.93 cm was recorded in karanj oil treated plots, and the untreated control resulted (3.7 cm) stem tunneling in comparison to bio-rational insecticides treated plots.

Grain yield (q ha^{-1})

The highest grain yield was recorded in emamectin benzoate 5% SG (40.97 q ha^{-1}) which was followed by spinosad 45% SC (34.33 q ha^{-1}), chlorantraniliprole 18.5% SC (32.14 q ha^{-1}), *Bacillus thuringiensis* (31.55 q ha^{-1}), azadirachtin 0.15% SC (30.96 q ha^{-1}), *Beauveria bassiana* 1.15 % WP (28.18 q ha^{-1}) and indoxacarb 14.5% SC (27.18 q ha^{-1}), respectively, while the lowest grain yield of 26.37 q ha^{-1} was recorded in karanj oil treated plots, and the untreated control resulted least (17.51 q ha^{-1}) grain yield in comparison to bio-rational insecticides treated plots.

Present findings are in accordance with the findings of DEOLE *et al*. (2017) who reported that spinosad 45 SC and chlorantraniliprole 18.5 SC were found effective against pink stem borer and recorded significantly superior grain yields of 62.71q/ha and 61.53 q/ha respectively with 45.16 and 42.43 per cent increased grain yields respectively. Similarly, RAMEASH *et al.* (2012) also reported unsatisfactory control of *C. partellus* in plots treated with entomopathogenic fungi *B. bassiana*. However, few earlier reports established the efficacy of these fungi against the maize stem borer under field conditions (GARDEZI *et al*., 1998; SHEKHARAPPA, 2001). RAMEASH *et al.* (2012) found satisfactory control of *C. partellus* in plots treated with spinosad (0.002%) and emamectin benzoate (0.002%). Apart from their bio-efficacy, the desirable qualities like low mammalian toxicity (WILLIAMS *et al*., 2003); safety to non-target organisms including natural enemies (Jyoti and Goud, 2008; SHARMA and KAUSHIK, 2010) and no cross-resistance with conventional insecticides (AHMED *et al*., 2002; DARRIET *et al*., 2005) make these natural insecticides as ideal alternatives for the ecofriendly management of *C. partellus* in maize

Economic estimation of different insecticides against pink stem borer of maize

The highest yield over control was obtained under treatment emamectin benzoate 5% SG (40.97 q ha^{-1}) which was followed by spinosad 45% SC (34.33 q ha^{-1}), chlorantraniliprole 18.5% SC (32.14 q ha^{-1}) and *Bacillus thuringiensis* (31.55 q ha^{-1}). Price of increased yield over control was calculated and highest price was with emamectin benzoate 5% SG (Rs.32022.90) lowest was with karanj oil (Rs.12093.90). Thus application of emamectin benzoate 5% SG, spinosad 45% SC and chlorantraniliprole 18.5% SC proved to be the best regarding management of pink stem borer and grain yield of maize.

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NEW RECORDS OF THE BEE GENUS GNATHONOMIA PAULY (HYMENOPTERA: APOIDEA: HALICTIDAE) FROM KERALA

MANJUSHA, K. T.[1] *AND JOBIRAJ, T.*[2]

ABSTRACT: Two species of *Gnathonomia thoracica* (Smith,1875), namely *Gnathonomia aurata* (Bingham, 1897), have been reported for the first time from Kerala state of India. These species have been diagnosed with the help of microphotographs.

KEY WORDS: *Gnathonomia*, Apoidea, Halictidae.

INTRODUCTION

The sub family Nomiinae (Halictidae: Hymenoptera) is cosmopolitan in distribution comprising over 500 species. Members of this family mostly occur in arid and semi arid zones. They are nest building, solitary (rarely sub social) bees (ASTAFUROVA, 2007). The striking features of these bees are three sub marginal cells in their fore wings, first and third cells sub equal in length while second shorter. Antennae arise near the middle of the eyes. Episternal groove present up to scrobe but sometimes a weak depression below scrobal groove. Legs of male Nomiinae are greatly modified which facilitate them during mating with female (WEISLO and BUCHMAN, 1995). There are 136 valid species of Nomiinae recognisd in Oriental region (PAULY, 2009) According to Pauly's Atlas Hymenoptera Online (2017), there are 22 genera of the sub family Nomiinae worldwide and in India it is represented by 15 genera and 72 species (SAINI & RATHORE, 2012). Among these 22 genera of Nomiinae reported in the world the genus *Gnathonomia* (PAULY, 2005) is endemic to oriental region. The reperesentatives of this genus are easily recognized by the oval tegulae which are of the 'auricle '(ear) form in *Curvinomia*. There are 10 species worldwide (Atlas Hymenoptera Online 2017, PAULY). Currently 3 species of this genus are known from India (SAINI and RATHORE, 2012).

MATERIALS AND METHODS

A survey was conducted for the collection of bees during 2015-2017. The various Bee species were collected from different parts of Kerala using sweep nets. The specimens collected were killed using insect killing jar containing ethyl acetate, followed by labeling and was pinned using entomological pins. The bees were then examined and identified using Leica EZ4HD Stereo Zoom Microscope. Illustrations of the identified specimens were also done using Leica EZ4HD Stereo Zoom Microscope and then preserved in wooden boxes containing naphthalene balls and para di chloro benzene. The entire research was carried out in the Zoology Laboratory, Government College Kodanchery, Kozhikode, Kerala. Specimens were identified using the keys suggested by Saini and Vikram (2012) and also Pauly (2009).

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RESULTS AND DISCUSSION

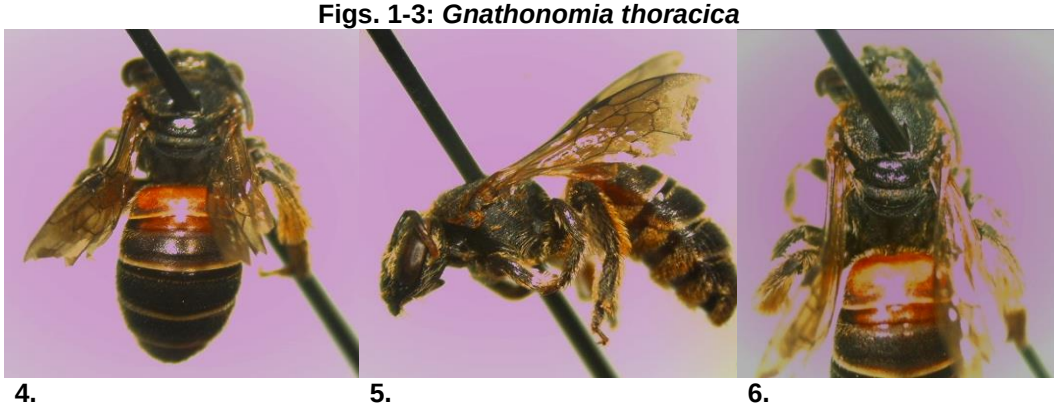
1. *Gnathonomia thoracica* (SMITH, 1875) (Figs.1-3)
Nomia albofasciata (SMITH, 1875)
Paranomia stantoni (ASHMEAD, 1904)
Nomia thoracica stantoni (ASHMEAD, 1904)
Nomia thoracica excellens homonym (COCKERELL, 1931)
Nomia melior (COCKERELL, 1931)

Diagnostic Characters
Head and mesonotum densely and finely, abdomen more minutely punctured; Scutellum prominent. Thorax completely covered with occluded felting; mandibles with a tooth on the inner edge and a hook pointing downwards; segments narrowly white; the flagellum of each antenna, except the basal two joints, fulvo testaceous; wings hyaline and nerves and tegulae testaceous

Materials examined
1♂, INDIA, Kerala, Thachampoyil, 29-VI-2015, Soumya K;1♀, INDIA, Kerala, Thamarassery, 6-XII-2016, Manjusha K T; 1♀, INDIA, Kerala, Thamarassery, 28-XII-2016

1 **2** **3**

Figs. 1-3: *Gnathonomia thoracica*



4. **5.** **6.**

Figs. 4-6 *Gnathonomia aurata*

Measurements (Male)
Body length: 10-12mm, Fore wing length &Width: 8.5-9mm, 3.5-4mm
Distribution: China, India (West Bengal, Sikkim**),** Indonesia

***2. Gnathonomia aurata (*BINGHAM, 1897) (**Figs. 4-6)
*Nomia aurata (*BINGHAM, 1897)
*Gnathonomia aurata (*BINGHAM 1897)
Nomia nasicana (COCKERELL, 1911)
Nomia crassiuscula (FRIESE, 1913)
Nomia perconcinna (COCKERELL, 1920)

Diagnostic Characters
Head and thorax somewhat coarsely and closely, abdomen minutely and very densely punctured; clypeus not carinate, flat, transverse anteriorly; the space of base finely reticulate. Abdominal segment 2-4 covered with cinereous; the legs with pale glittering pubescence; wings hyaline.

Materials examined
1♀, INDIA, Kerala, Manjusha K T, 28-XII-2016; 1♀ INDIA, Kerala, Manjusha K T, 23-XII-2016.

Measurements: Body length: 9-11mm, Fore wing length &Width: 6.5-7mm, 3.5-4mm

Distribution: India (Uttarakand), Burma, Thailand

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EFFECT OF DIFFERENT DIETS ON THE LIFE CYCLE OF *CORCYRA CEPHALONICA* STAINTON

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ABSTRACT: Studies conducted on the biology of rice moth, *Corcyra cephalonica* Stainton (Lepidoptera : Pyralidae) reared on ten different diets during July, 2016 to January, 2017 in the Biocontrol Laboratory, Department of Entomology, IGKV, Raipur (C.G.) revealed significant maximum and minimum larval weight in maize and Kodo respectively. Similarly, significant highest larval length was recorded in maize and lowest in kodo. The adult longevity of female was found significantly greater than male and highest longevity of female was found in rice and lowest in maize. Shortest life cycle of male and female was recorded in maize (44.66 and 46.66) and longest in rice of (67.00 and 71.00), respectively. Hence, maize as diet can be recommended to get adults of *C.cephalonica* in the shortest time while going of laboratory mass production.

Key words: *Corcyra cephalonica*, life cycle, maize, rice moth

INTRODUCTION

The rice meal moth, *Corcyra cephalonica* Stainton is being utilized in various bio-control research, developmental and extension units for mass production of number of natural enemies. It ranks first in the mass culturing of entomophagous insects due to its amenability to mass production, adaptability to varied rearing conditions and its positive influence on the progeny of the natural enemies. It also has been proved to be one of the most efficient surrogate hosts for rearing a wide range of biological control agents. The important among them are egg parasitoids – *Trichogramma* spp., egg larval parasitoids – *Chelonus blackburni*, larval parasitoids – *Bracon* spp., *Goniozus nephantidis*, *Apaneteles angaleti*, insect predators – *Chrysoperla carnea*, *Mallada boniensis*, *Cyrtorhynus feltiae*, and reduviid bug (*Rhynocoris* sp.) are reared on the larvae of *C. cephalonica* (JALALI and SINGH, 1992 and KUMAR and KUMAR, 2002). Besides, some entomopathogenic nematodes such as *Steinernema feltiae* are also reared on the larvae of *C. cephalonica* (KUMAR and MURTHY, 2000). Only an efficient and healthy insect mass rearing medium can result in mass production of effective biological control agents. Earlier studies showed that the combination of different diets can improve the ratio of female hosts amongst the insect mass. The present studies on the life cycle of *C. cephalonica* reared on ten different diets, including rice, maize, bajra, minor millets like kodo, kutki and ragi as solo and combination treatments was conducted in the bio-control laboratory, department of Entomology,IGKV, Raipur during July 2016-January 2017.

MATERIAL AND METHODS

Ten treatments of host diets (Table-1) were tested using three replications. The grains (625 gm) were placed in each plastic basin of size diameter (30 cm) height (10 cm). The grains were sterilized in hot air oven for one hour at 100^0C. After cooling the grains were powdered coarsely. 5 ml of 10% honey solution along with 5gm of yeast and a pinch of Streptomycin were mixed in each container. Finally the containers were charged with 0.25cc (about 4750 eggs) of *C. cephalonica.* Every container was covered with fine muslin cloth and secured tightly with help of plastic cord. Observations were recorded after two days onwards to note the hatching of the eggs.

Table-1: Treatments of host diets for rearing *C. cephalonica* under lab conditions

S. No.	Treatments
1	Rice (T1)
2	Maize (T2)
3	Bajra (T3)
4	Rice+Maize+Bajra (T4)
5	Kodo (T5)
6	Kutki (T6)
7	Ragi (T7)
8	Rice+Kodo+Kutki+Ragi (T8)
9	Maize+Kodo+Kutki+Ragi (T9)
10	Bajra+Kodo+Kutki+Ragi(T10)

A small representative samples from each treatment were kept separately in a small petridish to observe and record the size of the neonate larvae and the instar wise confirmation were done by looking at the shed head capsule. The length and weight of larvae were recorded per treatment. For studying the difference in fecundity a pair of newly emerged male and female from each treatment were kept in separate beakers in three replications. A cotton swab soaked in 10% honey solution was provided for moths stuck to the inner walls of beaker. Number of eggs laid by females was recorded everyday and also total number of eggs laid by a female reared on different diets per treatment was also computed**.**

RESULTS AND DISCUSSION

The experiment was conducted with seven different cereals as diets along with their combinations comprising of ten treatments. The cereals used were rice (*Oryza sativa*), maize (*Zea maize*), sorghum/bajra (*Pennisetum glaucum*), jowar/oat (*Sorghum bicolor*), kutki (*Picrorrhiza kurrora*), kodo (*Paspalum scorbiculatum*) and ragi (*Eleusine coracana*). Observations were recorded on the incubation period, length and weigth of the larvae were computed and analyzed under Completely Randomized design (C.R.D.) and presented in Table-2.

a) Incubation period: The results on incubation period obtained in the present study varied significantly between diets. The longest incubation period was found in T1 (Rice) 9 days followed by T5 (kodo) 8.33 days and shortest of 6 days was found in T2 (Maize), (Table-2).

b) Larval length: The results on larval length obtained in present study varied significantly within diets. In the first instar larval stage, the larval length did not vary significantly, but from 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instar onwards, significant difference were observed. Maximum and minimum larval length was observed in T2 (maize) of 2.92 mm and T5 (kodo) 2.16; T2 (maize) of 5.00 mm and T5 (kodo) of 3.00 mm; T2(maize) of 10.99mm and T5 (kodo) 7.55; T2 (maize) 13.67 mm T5 (kodo) 10.83mm in 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instar larval stages, respectively (Table-2).

(c) Larval weight: The results on larval weight obtained in present study also varied significantly within diets. In the 1st instar larvae the larval weight was highest in T2 (maize) (0.530 mg), and lowest in T5 (kodo) of (0.047 mg), followed by T8 (rice+kodo+kutki+ragi) (0.060 mg). In the 2nd , 3rd, 4th and 5th instar larval conditions the highest larval weight was found in treatment T2 (maize) with an average weight 1.26 mg, 8.66mg, 14.00mg and 30.33 mg respectively; where as lowest larval weights recorded larvae in T5 (kodo) of 0.56mg, 6.00mg, 7.33mg, and 10.00 mg in 2nd, 3rd, 4th and 5th instar, respectively.

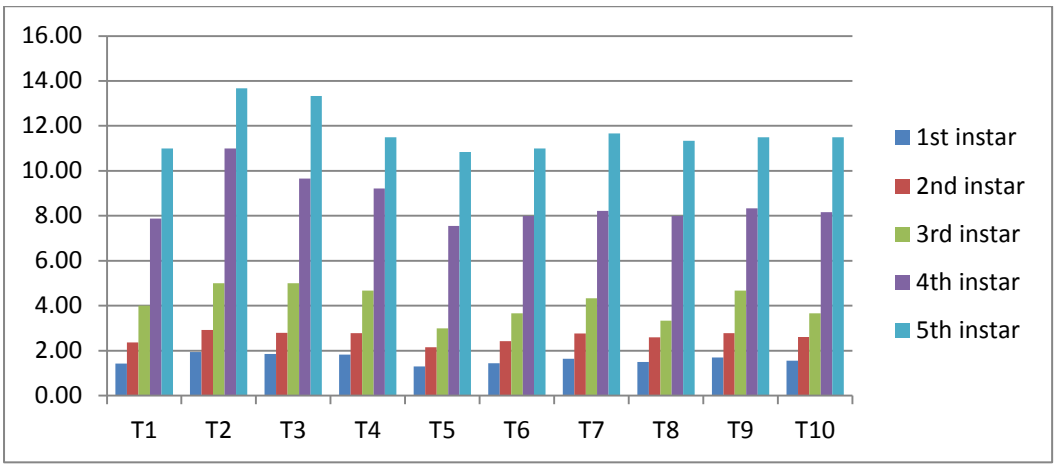

Fig-1: Graphical representation of larval length (in mm) of *C. cephalonica* at different instars fed on various diets.

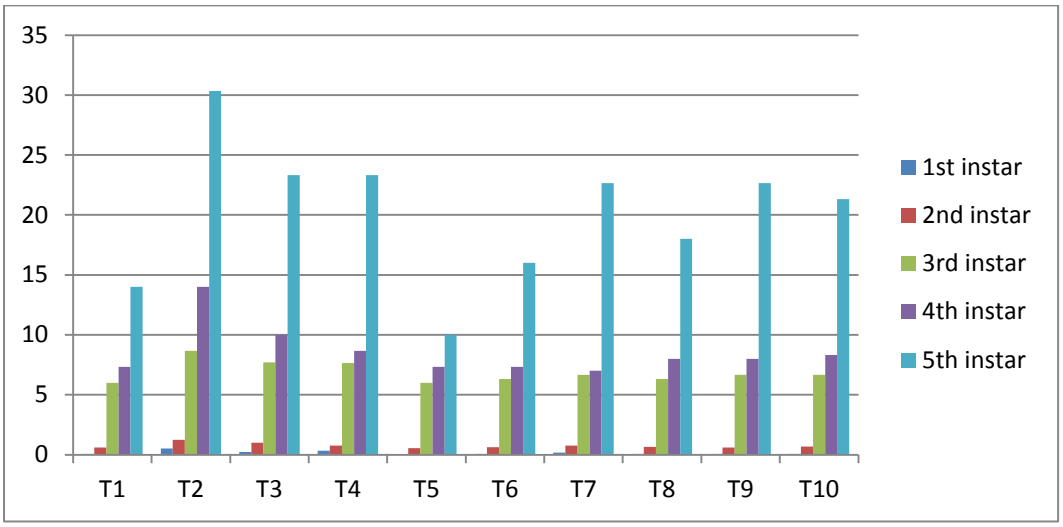

Fig.2: Graphical representation of larval weight (in mg) of *C. cephalonica* at different instars fed on various diets

Table-2: Incubation period, larval length (in mm) and larval weight (mg) of *C. cephalonica* at different instars fed on various diets

Treatment	**Incubation priod**	**1st instar**		**2nd instar**		**3rd instar**		**4th instar**		**5th instar**	
		Length	**Weight**	**Length**	**Weight**	**Length**	**Weight**	**Length**	**Weight**	**Length**	**Weight**
T1	9.00	1.42	0.070	2.37	0.60	4.00	6.00	7.88	7.33	11.00	14.00
T2	6.00	1.97	0.530	2.92	1.26	5.00	8.66	10.99	14.00	13.67	30.33
T3	7.00	1.86	0.230	2.80	1.00	5.00	7.70	9.66	10.00	13.33	23.33
T4	7.00	1.82	0.353	2.78	0.76	4.67	7.66	9.22	8.66	11.50	23.33
T5	8.33	1.27	0.047	2.16	0.56	3.00	6.00	7.55	7.33	10.83	10.00
T6	7.00	1.45	0.067	2.43	0.63	3.67	6.33	8.00	7.33	11.00	16.00
T7	7.00	1.64	0.167	2.77	0.76	4.33	6.66	8.22	7.00	11.67	22.66
T8	8.00	1.50	0.060	2.60	0.66	3.33	6.33	8.00	8.00	11.33	18.00
T9	6.00	1.69	0.063	2.78	0.60	4.67	6.66	8.33	8.00	11.50	22.66
T10	6.00	1.55	0.070	2.60	0.70	3.67	6.66	8.16	8.33	11.50	21.33
SEm±	0.053	0.11	0.015	0.14	0.036	0.25	1.12	0.37	0.43	0.36	0.88
CV	1.28	12.01	17.43	9.77	22.10	10.82	31.44	7.60	8.75	5.33	7.65
CD at 5%	0.157	0.45	0.045	0.43	0.10	0.76	3.33	1.11	1.28	1.06	2.63

(d) **Total life cycle of *C. cephalonica* reared under different diets**

The shortest life cycle of male and female both were found in Maize (T2) in table no. 3 (44.66 and 46.66 days respectively) and the longest life cycle was seen in Rice (T1) (67.00 and 71.00 days respectively). Similar findings were reported by BHARDWAJ *et al*., 2016, in which among the thirteen different solo and combination treatments tested for rearing *C. cephalonica* under laboratory conditions, the longest life cycle was recorded on rice of 60 to 70 days.

Table-3: Duration of life cycle of *C. cephalonica* reared under different diets

Treatment	Incubation period (days)	Larval period (days)	Pupal period (days)	Longevity (days)		Total days	Total days
				Male	Female	Male	Female
T1	9.00	40.00	14.00	4.00	8.00	67.00	71.00
T2	6.00	26.66	10.00	2.00	4.00	44.66	46.66
T3	7.00	28.00	10.33	2.00	4.33	47.33	49.66
T4	7.00	30.01	10.66	2.00	4.16	49.67	51.83
T5	8.33	37.83	3.00	3.00	8.00	52.16	57.16
T6	7.00	36.33	12.00	2.00	7.00	57.33	62.33
T7	7.00	36.66	11.00	2.00	5.00	56.66	59.66
T8	8.00	34.66	13.00	2.00	6.00	57.66	61.66
T9	6.00	33.33	12.00	2.00	5.00	53.33	56.33
T10	6.00	33.66	12.66	2.66	6.00	54.98	58.32
SEm±	0.053	0.83	0.73	0.21	0.11		
CV	1.28	4.30	10.65	10.61	3.55		
CD at 5%	0.157	2.48	1.03	0.44	0.35		

e) B: C ratio: Economics for the mass production of *C. cephalonica* under laboratory conditions was estimated for solo and combination diets as mentioned in Table-4.

Table-4: Cost of grains used in experiment for different solo and combination diets

Name of cereals	Price of cereals/ kg (in Rupees)	Treatment	Cost of 625 gm. grains
Rice	18.00	Rice (T1)	11.25
Maize	30.00	Maize (T2)	18.75
Bajra	26 .00	Bajra (T3)	16.25
Jwar	30 .00	Rice+Maize+Bajra (T4)	15.41
Kutki (chidiya dana)	70 .00	Kodo (T5)	28.12
Kodo	45 .00	Kutki (T6)	43.75
Ragi	28 .00	Ragi (T7)	17.50
		Rice+Kodo+Kutki+Ragi (T8)	25.14
		Maize+Kodo+Kutki+Ragi (T9)	27.01
		Bajra+Kodo+Kutki+Ragi (T10)	26.39

The prices of grains are mentioned in above table based on 625 gm of grains as in each solo and the combination treatment contained equal amount of each cereal. The treatment T2 (maize) performed best as far as the biological parameters were concerned but cost wise, it was higher than rice. But looking to the duration of life cycle it was 1.52 times faster in maize as compared to rice. The performance of *C. cephalonica* reared on solo rice was found to be the poorest in all parameters of life cycle studied in the present investigation. Thus, rearing of *C. cephalonica* on maize can be suggested for faster production of *Corcyra* larvae on commercial scale. BHARDWAJ (2016) also reported the cheapest for mass rearing *C. cephalonica* in the laboratory was maize (9.00/- price of 600 gm. grains) among the thirteen diet treatments tested including solo and combination grains.

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ARTHROPOD BIO-CONTROL AGENTS OF WATER HYACINTH RECORDED AT RAIPUR, CHHATTTISGARH

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ABSTRACT: The aquatic weed water hyacinth, *Eichhornia crassipes* Martius poses several problems in India and all over the world. Insects as major bio-agent have been successfully utilized world over for managing the weed. Five arthropod species were recorded as pests for the first time in Chhattisgarh during the present studies. Both of the *Neochetina* weevil species namely *Neochetina eichhorniae* and *N. bruchi* were recorded at two ponds during the study conducted at three sites Tikrapara, Mandir Hasaud and Fundhar. *Neochetina* species was not recorded at Mandir hasaud. Weevil abundance varied among sites and between species. *N. eichhorniae* was significantly more abundant than *N. bruchi* at Tikrapara pond. Other arthropod species recorded were two species of water hyacinth mite, *Galumna* spp.(Acari: Galumnidae), *Tetranychus urticae* Koch (Acari: Tetranychidae), aphid, *Rhopalosiphum nympheae* (L.) (Hemiptera: Aphidae), the water hyacinth grasshopper, *Cornops aquaticum* and one unidentified Lepidopteran insect.

Key words: water hyacinth, arthropod, *Neochetina* weevil.

INTRODUCTION

Water hyacinth, *Eichhornia crassipes* Martius is an exotic free floating perennial aquatic herb native to South America belonging to the family Pontederiaceae, closely related to the Liliaceae (lily family). Originally introduced in 1884 for its beautiful flowers, water hyacinth has invaded most of the southern United States and many tropical and sub tropical regions around the world. JULIEN and GRIFFITHS (1998) The mature plant consists of long, pendant roots, rhizomes, stolons, leaves, inflorescences and fruit clusters. The plants grow up to 1 metre high although 40cm is the more usual height. The inflorescence bears 6 - 10 lily-like flowers, each 4 - 7cm in diameter. The stems and leaves contain air-filled tissue which give the plant considerable buoyancy. The vegetative reproduction is asexual and takes place at a rapid rate under preferential conditions. It displaces native vegetation due to its rapid growth during summer. Major economic impacts caused by invasion of water hyacinth include interference with navigation, irrigation and power generation. Additionally, dense mats can provide ideal mosquito breeding habitats. SUSHIL KUMAR (2011) ranked water hyacinth, *E. crassipes* on the top of the list of about 160 aquatic weeds of primary concern in India.

Several biological control agents have been introduced in to more than 30 countries having problems with water hyacinth, *E. crassipes*, and they are contributing to weed control for many areas. The host-specific weevils *Neochetina eichhorniae* and *N. bruchi* are the leading and most successful biological agents used for the control of *E. crassipes* (JIMENZE *et al.,* 2001). Among them *N. bruchi* is the most widely used species to control *E. crassipes*. It is a weevil with four development stages of its life cycle, eggs, larvae, pupae and adult (ELAWELLA *et al.,* 2007). In the present studies conducted at three different ponds of Raipur district, Chhattisgarh state during 2016-17, *Neochetina eichhorniae* and *N. bruchi* were recorded as the main insects feeding on the foliage of water hyacinth. Apart from these, grasshopper, *Cornops aquaticum* aphids,

Rhopalosiphum nympheae two species of water hyacinth mites, *Galumna* spp. (Acari: Galumnidae), *Tetranychus urticae* Koch (Acari: Tetranychidae), were also seen feeding and damaging in the leaves of the aquatic weed.

MATERIALS AND METHODS

The present experiment was conducted at three local ponds of Raipur district *viz*., Tikrapara pond, Mandir Hasaud pond, and Fundhar pond during 2016-17. Observations were recorded at fortnightly interval at the three above mentioned local ponds mainly on the number of insects of different spp. on the leaves, stem, flower and roots of ten plants randomly selected plants from each pond. Qualitative monitoring of water hyacinth and *Neochetina* weevil was carried out every month from July 2016 to February, 2017 on the above mentioned ponds.

RESULTS AND DISCUSSION

Assessment of weevil population was done by counting the number of *N eichhorniae* and *N. bruchi* adult weevils separately from each of the ten plants randomly selected at each site. Larval and pupal populations were not assessed. The population of other insects were also recorded i.e. aphid, *Rhopalosiphum nympheae* (L.) and two species of water hyacinth mites, *Galumna* spp. (Acari: Galumnidae) and *Tetranychus urticae* Koch (Acari: Tetranychidae). Pierce wounds were present, indicating that sucking pests such as aphids and streak were formed due to mites feeding on the plants. The mite, *Tetranychus urticae* was found feeding on the upper surface of the leaves, while *Galumna* spp. was found feeding inside leaf tissues. All the insects and mites were identified at NBAIR, Bangalore. Mean population of the bioagents recorded during the study is represented in Table-1.

Table-1: Mean population of various insects recorded from different ponds during July 2016 - February 2017

S.No.	**Tikrapara**			**Mandir hasaud**		**Fundhar**		
	Insects	**Total**	**Mean**	**Total**	**Mean**	**Total**	**Mean**	**Overall mean**
1	*N. bruchi*	175	11.67	0	0	73	16.22	9.29
2	*N. eichhorniae*	184	12.27	0	0	0	0	4.09
3	grasshopper	48	3.2	52	6.5	0	0	3.23
4	aphid	0	0	0	0	1644	365.33	121.77
5	Mites	12018	801.2	0	0	0	0	267.06

Initially all the three sites infested with water hyacinth had populations of *Neochetina* weevils; however, there was a lower weevil infestation in Fundhar pond. The highest mean number of the weevil, *N. bruchi* per plant was16.22 at Fundhar pond and highest mean number of weevil, *N. eichhorniae* per plant was12.27 at Tikra para pond.

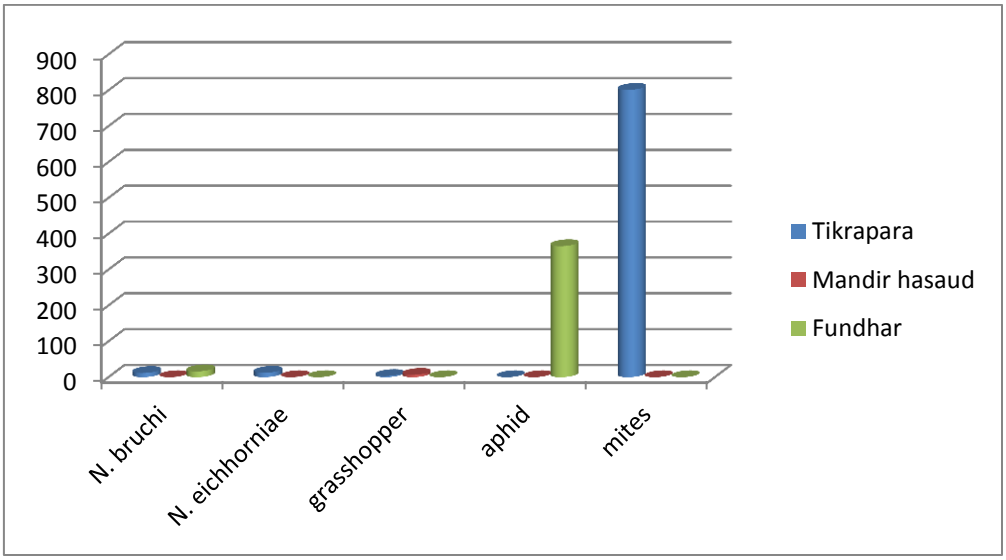

Fig.1: Graphical representation of mean population of various insects recorded from different ponds during July2016 – Feb 2017

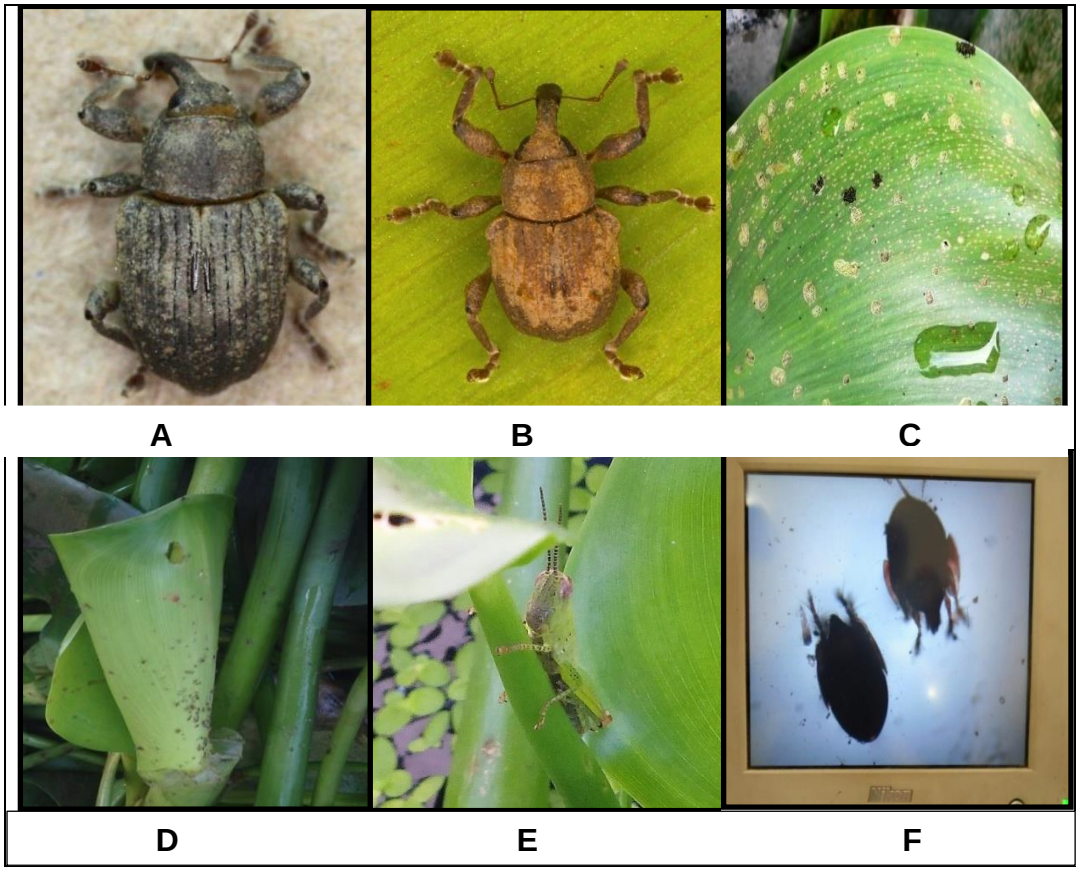

Photo-1: A= *N. eichhorniae*, B= *N. bruchi*, C= Mites, D= Aphid, E= Grasshopper, F= Magnified view of mite, *Galumna.* sp.

Lowest mean number of weevil, *N. bruchi* per plant 11.67 was also recorded at Tikrapara pond. Overall mean population of the weevil indicate that *N. bruchi* was found to be higher than *N. eichhorniae* (Table-1.) which is in accordance with RAY and SUSHIL KUMAR (2015), who also reported that *N. bruchi* dominated in comparison to *N. eichhorniae* at Jabalpur, M.P. The density of the weevils (i.e. the number of weevils per plant) determines the level of damage that is inflicted on the plant by the weevils. The higher the weevil density, the greater was the impact of the weevils on the plants. For significant impacts the weevils must establish and their numbers must increase to thresholds that will impact negatively on the water hyacinth population build-up. At the site Tikrapara pond, highest mean number of arthropod pest was mite followed by *N. eichhorniae*, *N. bruchi* and Grasshopper. No aphids were recorded from this site. At the site Mandir Hasaud pond only Grasshopper, *Cornops aquaticum* was recorded. From Fundhar pond the highest mean number of insect found was aphid and the lowest mean number of insect recorded was *Neochetina bruchi*. Other insects were not recorded from this pond.

Thus present studies can be concluded that water hyacinth plants in Raipur were fed on by several arthropods. Two species of water hyacinth weevils, *Neochetina bruchi* and *N. eichhorniae,* two species of mites, *Galumna* spp. (Acari: Galumnidae) and *Tetranychus urticae* Koch (Acari: Tetranychidae), aphid, *Rhopalosiphum nympheae* (L.) (Hemiptera: Aphidae), and water hyacinth grasshopper, *Cornops aquaticum* and one unidentified Lepidopteran insect were recorded as pests feeding on water hyacinth plants. Based on the abundance, these arthropods can be further studied and exploited as effective bio-control agents for eco-friendly management of the noxious aquatic weed.

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PREVALENCE OF MONOGENEAN PARASITES ON GREY MULLETS IN KAYAMKULAM ESTUARY, KERALA, INDIA

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ABSTRACT: In the present study, 300 grey mullets were collected from the Kayamkulam estuary, south west coast of Kerala for the detection of monogenean fish parasites during the period of February 2015 to January 2016. Out of the 300 host fishes, 65 fishes were infested with parasites. Three species of monogeneans were encountered from the grey mullets: *Ligophorus* sp., *Dactylogyrus* sp. and *Metamicrocotyla* sp. The parasitological terms such as prevalence, mean intensity and mean abundance were calculated to analyze the level of infection on the host fishes. The prevalence, mean intensity and mean abundance was 21.67, 2.91 and 0.63, respectively. Seasonal prevalence, mean intensity and mean abundance of infection were also noted. All the parasitological parameters showed higher value in post monsoon period and lower values on the monsoon months. The infection level of fishes with monogeneans was very low during the study period.

Key words: Grey mullet, monogenean parasite, *Ligophorus* sp., *Dactylogyrus* sp.

INTRODUCTION

The grey mullet is an important food fish in the family Mugilidae. Parasitic diseases of fishes are very common all over the world. Among the parasites, monogeneans are very important and cause damage to the host fishes in several ways. Monogenean parasites are found on fresh water and marine water fishes. The monogenetic trematodes are hermaphroditic flatworms that complete their life cycle on a single host and they mostly live as ectoparasites on the gills or general body surface of fishes (CABLE *et al*., 1999). A major identifying characteristic of monogenean parasite is their organ of attachment (the haptor). Most species descriptions of parasitic worms are based on morphology, but they also contain important information about ecology, including for example host, life history or locality (AL-ZUBAIDY, 2007). The viviparous monogenean fish parasite genus *Gyrodactylus* is one of the most specious genera among metazoan animals (CRIBB *et al*., 2000). The hook-like structures of monogeneans are used to attach to the fish. Monogeneans may cause severe damage in hatcheries of fish farms and may cause mortality in the wild (OBIAKEZIE and TAEGE, 1991). Monogenean infestations cause irritation and excessive mucus production and create an opening for bacterial invasion. A few monogeneans on a healthy mature fish are not usually significant; however, moderate numbers can cause significant mortalities. When fishes are exposed to environmental or behavioral stressors, the potential damage from monogeneans is greater. The present article is aimed to contribute on the prevalence of monogenean parasites on grey mullets in Kayamkulam estuary, Kerala, India.

MATERIALS AND METHODS

The study area, Kayamkulam backwater is a shallow brackish water lagoon. Kayamkulam backwater occupies area in both Alapuzzha district and Kollam of the total 1,652,33 hectares. Three stations were selected within the estuary. During the period

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from February 2015 to January 2016, host fishes were collected from the local fishermen. A total of 300 fishes were examined for the presence of monogeneans. Scraping from the skin, fins and operculum of the host fishes were done for the detection of parasites. After dissection, stereo-microscopic observation was made on gills for the presence of parasites. Parasitic identification was done on the basis of some major taxonomical references (BYKHOWSKAYA-PAVLOVSKAYA *et al.,* 1962; GUSEV, 1965; KHOTENOVSKY, 1985). The parasite number and place of their attachment were recorded. The collected parasites were fixed in glacial acetic acid and preserved using glycerin–gel under the cover slip (PRITCHARD AND KRUSE, 1982). The parasitological terms such as prevalence, mean intensity and mean abundance following BUSH *et al.* (1997) and MARGOLIS *et al.* (1982).

RESULTS AND DISCUSSION

The findings of the study in terms of the prevalence, mean intensity and mean abundance of monogenean infection are given in Table-1. A total of 300 grey mullets were analysed for monogenean parasites. Out of the 300 host fishes, 65 fishes were found infested by monogenean. The prevalence of infestation was highest (26%) during the post monsoon period and lowest level of prevalence (18%) noted on the monsoon periods. The higher value of mean intensity was 3.2 and lower value was 2.3.Regarding the mean abundance, the (0.84) was reported in the post monsoon and minimum value (0.42) was in the monsoon season (Table-1 and Fig.-1). Three species of monogeneans were recovered from the host fish grey mullet: *Ligophorus* sp*, dactylogyrus* sp and *metamicrocotyla* sp.

Grey mullets collected from Station III harbored three species of monogeneans. In post monsoon, *Ligophorus* sp infection was found on the grey mullets collected from all the three stations. *Metamicrocotyla* sp was found only on the grey mullets collected from station III during monsoon period (Table-2).

Table-1: The prevalence, Mean Intensity and Mean Abundance of Monogenean parasites on grey mullets

Seasons	Prevalence (%)	Mean Intensity	Mean Abundance
Pre monsoon	21	3	0.63
Monsoon	18	2.3	0.42
Post Monsoon	26	3.2	0.84
Total	21.67	2.91	0.63

The majority of the monogeneans showed higher prevalence, mean intensity and mean abundance in the post monsoon period (Fig-1). The lowest values of infection were recorded on the monsoon months. Egg development of monogeneans depends on temperature. Among monogeneans, *Dactylogyrus* species are more peculiar due to their high tolerance to a wide range of temperature and salinity. Monogeneans feed on epithelial cells, mucus and blood, and can cause damage including hemorrhage and ulceration of host epithelium out growth development and excessive mucus production which can disturb the respiratory function of the gills and ionic exchange (CHAPMAN *et al*., 2000). In the present, the host fishes were slightly infested with monogenean parasites. The prevalence, mean intensity and mean abundance were low throughout the study period.

Table-2: Monogenean parasites found in grey mullets of Kayamkulam Estuary

Monogenean	Station I	Station II	Station III
1. ***Ligophorus* sp.**			
Pre monsoon	+		+
Monsoon		+	
Post monsoon	+	+	+
2. ***Dactylogyrus*** sp.			
Pre monsoon			
Monsoon		+	
Post monsoon		+	+
3. ***Metamicrocotyla*** sp.			
Pre monsoon			
Monsoon			+
Post monsoon			

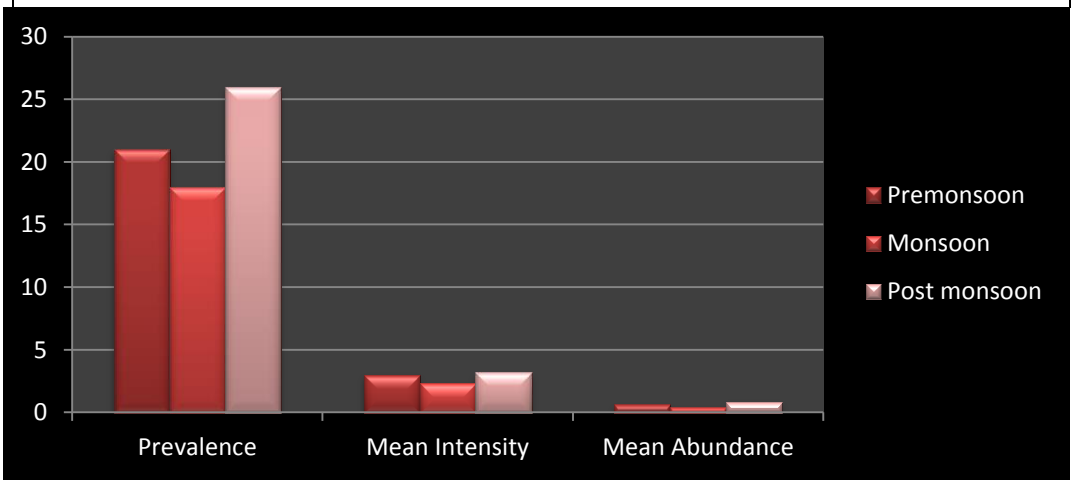

Fig.-1: Infestation prevalence, mean intensity and mean abundance levels of Monogeneans from the grey mullets

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SPIDER FAUNA OF BRINJAL ECOSYSTEM IN COASTAL ODISHA

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Received: 12 June, 2007 **Accepted 25 August, 2017**

ABSTRACT: The spiders associated with brinjal ecosystem in coastal Odiha have been studied during winter, 2012-13 in the experimental field with brinjal cv. Blue star. Eleven species of spiders represented by eight families have been documented. Spider family Oxyopidae predominated in brinjal ecosystem which was followed by the family Tetragnathidae, Dictynidae and Theridiidae. *Oxyopes birmanicus*, *Tetragnatha javana*, *Dictyna* sp., *Theridula angula* and *Thomisus* spp. were assessed as predominant spider species in brinjal ecosystem of coastal Odisha. Nutrient sources did not influence their population density appreciably, while insecticides showed comparatively less population of spiders (0.4/1 meter row length) than other treatments including control (1.20-1.70/1 meter row length).

Key words: Spiders, brinjal ecosystem, coastal Odisha, nutrient sources, insecticides

INTRODUCTION

Spiders constitute an essential portion of the predatory arthropods in several agro-ecosystems (SUNDARARAJ, 2008; GHAVAMI *et al*., 2008; CHATTERJEE *et al*., 2009) and they limit the exponential growth of pest populations in various ecosystems by virtue of their predatory potency (GHAVAMI, 2008; CHAKRABORTY *et al*., 2015). Although spiders may be more sensitive to insecticides than insects (RAVI *et al*., 2008), but some spiders show tolerance, perhaps even resistance, to some pesticides (TANAKA *et al.,* 2000). The insect pests on vegetables have been well studied; however the spiders received little attention in vegetable fields (VAYSSIERES *et al*., 2001). Thus, attempts were made in the present studies to document the spiders prevalent on brinjal crop and to ascertain the impact of bio-nutrients and safe insecticides on spiders under field conditions.

MATERIALS AND METHODS

The documentation of spiders was done during winter, 2012-13 in the experimental field with brinjal cv. Blue star intended to test the recommended dose of fertilizers (RDF) *i.e*. N: P_2O_5: K_2O @ 125:80:100 Kg/ha and 50% RDF + Bio-NPK in main plots and treatments *viz*., (1) Mixture of cow urine(10%) + cow dung(10%) + neem leaves (5%) **,** (2) Mixture of **c**ow urine+ cow dung (10%)+karanj leaves (5%) **(**3) Pot mixture of botanicals, (4) Spinosad 45 SC (1ml/lit water) (5) Carbosulfan 25 EC (2ml/lit. water) and (6) Untreated control in sub-plots, against the shoot and fruit borer, *L. orbonalis*. There were 12 treatments which were replicated thrice in split-plot design. The spider population / one meter row length was assessed from 3 randomly selected spots in each plot. The total spider population from the three 1 m row length was used to compare the impact of treatments and the nutrient levels. The spider samples collected during field observations were transferred in 70% alcohol. These samples were identified by examining them under Stereo zoom Binocular microscope and by comparing with TIKADER (1987). The website (http://www.southindianspiders.org/south-indian-spiders3.htm) was also referred as and when necessary. A few specimens were sent to

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Prof. Ganesh N. Vankhede, S.G. Amravati Univeristy, Maharastra for confirmation of the genus.

RESULTS AND DISCUSSION

Different spider species were invariably recorded in almost all plots irrespective of treatments. About 11 species of spiders represented by eight families were found associated with the brinjal crop during winter 2012-13 (Fig. 1). In proportion to the total spiders the family: Oxyopidae predominated (86.5%) in brinjal ecosystem during the above period of investigation and this was followed by the family Tetragnathidae (3.9%), Dictynidae (3.3%) and Theridiidae (3.1%) (Table1). The rest of the families like Salticidae and Lycosidae from non-web spinner group and Thomisidae and Araneidae from web spinner group constituted only a small proportion of 0.6 to 0.9%.

Table 1. Spider species associated with brinjal ecosystem during winter 2012-13

S.No.	Family (Proportion in %)	Species documented (Proportion in %)
Non-Web spinners		
1.	Oxyopidae (86.5)	Lynx spider , *Oxyopes birmanicus* Thorell, 1887 (91.6)
2.		Green spider, *Peucetia viridana* Stoliczka, 1869 (0.4)
3.	Tetragnathidae (3.9)	*Tetragnatha javana* (Thorell, 1890) (3.9)
4.	Salticidae (0.6)	Silver spider or Jumping spider, *Telamonia* sp (0.6)
5.	Lycosidae (0.7)	*Lycosa* spp. (0.2)
Web spinners		
6.	Theridiidae (3.1)	Comb footed spider, *Theridula angula* Tikader,1970 (3.0)
7.		*Theridion* sp. (0.1)
8.	Thomisidae (0.9)	Crab spider, *Thomisus* spp (0.9)
9.	Dictynidae (3.3)	*Dictyna* sp. (3.3)
10	Araneidae (0.7)	Orb web spider / garden spider, *Araneus* sp. (0.6)
11		White orb web spider, *Argiope* sp. (0.1)

Among the spiders species identified, the Lynx spider, *Oxyopes birmanicus* predominated constituting 91.6% of the total spiders documented during field observations and the proportion of other spider species in descending order of their predominance were *Tetragnatha javana* (3.9%), *Dictyna* sp (3.3%), *Theridula angula* (3.0%) *Thomisus* spp (0.9%) Jumping spider *Telamonia* sp and orb web spider, *Araneus* sp (0.6% each), *g*reen spider, *Peucetia viridana* (0.4%) and comb footed spider, *Theridion* spp and white orb web spider *Argiope* sp. (each 0.1%). However, SAKHINETIPALLI and PATNAIK (2013) observed about 9 species of spiders represented by 5 families in brinjal field and the lynx spiders (Family: Oxyopidae) alone consisted of five species, four belonging to the genus *Oxyopes* and one to the genus *Peucetia.* In Tamil Nadu, SANKARI and THIYAGISEN (2010) while studying the population of spiders and their predaceous potential in brinjal and snake gourd fields recorded 8 species of spiders. However, earlier RAJESWARAN *et al*. (2005) indicated that species diversity varied with different crop ecosystems and the authors have recorded maximum of 57 species in sugarcane and the crop ecosystems with descending species richness were

Non-Web spinners

***Oxyopes birmanicus* Thorell, 1887**

***Peucetia viridana* Stoliczka**

***Tetragnatha javana* Thorell**

***Telamonia* sp**

***Lycosa* spp.**

Web spinners

***Theridula angula* Tikader**

***Theridion* sp.**

***Thomisus* spp**

***Dictyna* sp.**

***Araneus* sp.**

***Argiope* sp.**

Fig.- 1: Spiders associated with brinjal crop at Bhubaneswar during winter 2012-13

reported as coconut (26 species), cotton (21 species), rice (19 species), oil seeds (18 species), soybean (16 species), maize (13 species), vegetables (13 species), fruit crops (11 species).

The nutrient level tested could not influence spider population appreciably and their population density remained uniform (1.03/ 3m row length) in plots received with RDF and 50% RDF+Bio-NPK (Table-2). While distinct variation in spider population density (0.40-1.70/3 m row length) have been recorded in response to various control strategies. It was observed that application of insecticide had affected its population and comparatively low population of 0.4/3 m row length was recorded in plots treated with either spinosad or carbosulfan.

The ITK based treatments like CU+CD+NL, CU+CD+KL and pot mixture showed reasonably high spider population density of 1.20-1.25 / 3m row length indicating their safety towards this predatory arthropods. Such densities of spiders in plots treated with ITK based treatments were also found on a par with that of untreated control plots which recorded maximum spider population of 1.70 /3 m row length. Thus, it was concluded that ITK based strategies are quite safe to the spiders and the documentation of different spider populations in brinjal ecosystem appeared to be the first report as no such efforts were made in the past from Odisha.

Table-2: Status of spider incidence on brinjal in response to nutrient sources & control

Treatment	Spider population (Mean No./1m row length)
Nutrient levels(main plot):	
RDF	1.03(1.20)
50%RDF + Bio NPK	1.03(1.21)
SE(m)±	0.01
CD (P=0.05)	ns
Control strategies (sub-plot):	
1.CU+CD+NL	1.20(1.28)
2.CU+CD+KL	1.25(1.32)
3.Pot mixture	1.25(1.30)
4.Spinosad45SC	0.40(0.92)
5.Carbosulfan25EC	0.40(0.95)
6.Control	1.70(1.48)
SE(m)±	0.03
CD(P=0.05)	0.09
Interaction:	
SE(m)±	0.04
CD(P=0.05)	0.12

* data average of 6 observations; [1]Figs.in parentheses are √x+0.5 transformed values; ns: not significant
CU = Cow urine, CD = Cow dung, NL = Neam leaves, KL = Karanj leaves

Strategies to conserve and enhance spider population are felt imperative to reduce insect pest menace in brinjal crop ecosystem. Moreover, the use of spiders as biocontrol agents offers a safe alternative to use of chemical pesticides (UMARANI and UMAMAHESWARI, 2013). Straw shelters have been used by Chinese farmers for >2 000 years to provide temporary spider refugia during cyclic farming disturbances (USDA, 1982). As such the straw bundles (90 cm in length and 25 cm in diameter) prepared with

wheat straw and placed in plastic nets were successfully utilized for augmentation of spiders in rice crop (TANWAR *et al*., 2008). The above technology has now been recommended by NCIPM as an important component of IPM in rice (TANWAR *et al*., 2011). The possibility of augmenting spider population in brinjal crop with such technique is also foreseen.

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REDISCOVERY OF *GOBIUS MALABARICUS* DAY FROM ITS TYPE LOCALITY AFTER ONE AND A HALF CENTURY

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ABSTRACT: *Gobius malabaricus* Day was considered partly as a synonym of *Stenogobius* species; but no specimen of it was collected from its type locality after its description by Francis Day in 1865. Four specimens of *Gobius malabaricus* Day were collected from its type locality and it is assigned to the appropriate taxa.

KEY WORDS: Rediscovery, *Gobius malabaricus, Stenogobius macropterus*

INTRODUCTION

Gobius malabaricus was described by DAY (1865a, 1865b, 1877, 1899) from Karavannoor River of Kerala. He described it distinctly and wrote of it as 'common in Kurriavannoor River north of Cochin, when freshes from the ghauts rush down to the sea'. But no specimens of it were collected from its type locality after the last 150 years. But the name *Gobius malabaricus* still exists in scientific literature partly as synonyms of other species. Great confusion and taxonomic ambiguity existed in its identity. It was synonymysed with *Stenogobius gymnopomus* (Bleeker 1853) by TALWAR and JHINGRAN (1991) and *Stenogobius macropterus* (Duncker 1912) by WATSON (1991). Species of the genus *Stenogobius* are limited to freshwater and marine conditions. As there had existed a taxonomic ambiguity surrounding its classification, the identity of the genus was not certain in past. So it was included by many in the genus *Awaous* Valenciennes (1837). *Stenogobius* can be identified from other genera in having detached and rudimentary gill rakers, terminal lower jaw, truncate glossohyal and oblong and pointed caudal fin. They can further be distinguished by the absence of papillae on gill structure, interior of gill cover and on palate. During a collection of fish from Trichur district of Kerala, 4 specimens of *Stenogobius* fishes were collected; after careful taxonomic analysis it was confirmed that those were nothing but the *Gobius malabaricus* of DAY (1865a, b).

MATERIALS AND METHODS

Methods used are those of JAYARAM (2002). Measurements were made point to point with dial calipers and data recorded to tenths of a millimeter. Counts and measurements were made on the left side of specimens. Subunits of the head were presented as proportions of head length (HL). Head length and measurements of body parts were given as proportions of standard length (SL). Distance between two fins or between fin and vent is taken from the origin of the fin. The new fish is deposited in museum of Zoological Survey of India (ZSI / SRC) at Chennai, India.

RESULTS AND DISCUSSION

Topotypic specimens examined: ZSI/SRC **F- 9151**, 4 exs, 66.00- 85 mm SL, Karavannoor River at Aarattupuzha, Collected by Dr. Mathews Plamoottil, 02.03.2015.

Diagnosis: Many vertical bands present on sides and back. Second dorsal, anal and caudal fin reddish brown. Dorsal fin with vi, i, 10 rays; pectoral fin with 14- 15 rays; anal fin with i, 10 rays. 50 scales present on lateral series. Second dorsal, anal and caudal fin bears distinct black spots. Blackish markings present on upper pectoral base and under gill cover. A conical dark band runs directly downwards from eye to lower edge of preopercle. Dorsal fin has a black crescentic mark at its base bordered by curved white and black bands.

Table-1: Morphometric characters of *Gobius malabaricus*

S. No.	**Characters**	**Range**
1	Total Length (mm)	95.0- 110.0
2	Standard Length (mm)	66.0- 85.0
3	Head Length (mm)	17.0- 22.0
	% Standard Length	
4	Head Length	22.7 – 25.9
5	Head Depth	18.6 – 18.
6	Head Width	20.0
7	Body Depth at Dorsal fin Origin	17.6 – 20.0
8	Body width at dorsal fin Origin	16.0 – 17.6
9	Pre Dorsal Distance	32.9 – 34.7
10	Post Dorsal Distance	68.7 - 70.6
11	Pre Pectoral Distance	25.9 – 26.7
12	Pre anal Distance	55.3 – 58.7
13	Height of Dorsal fin	21.3 – 28.2
14	Length of Pectoral Fin	20.0 – 22.7
15	Length of Caudal fin	29.4- 33.3
16	Length of Base of Anal fin	25.8- 27.3
17	Length of Caudal Peduncle	20.0
18	Depth of Caudal Peduncle	9.4- 12.0
19	Distance from Pelvic to Anal fin	29.4- 32.0
20	Distance from Anal to Caudal fin	46.6- 47.1
21	Head Length (mm)	17.0- 22.0
	% Head Length	
22	Head Depth	
23	Head Width	
24	Eye Diameter	25.0- 29.4
25	Inter Orbital Width	22.4- 22.7
26	Snout Length	29.4- 31.8
27	Width of Gape of Mouth	38.2- 45.5

Description: Body elongate, subcylindrical and slightly compressed; mouth terminal, lower jaw not protruding and slightly oblique, upper jaw protractile; opercle and preopercle without spines and edges smooth, subcylindrical and slightly compressed. Maxilla extends backwards to front border of orbit; moderately strong crest present on nape region. Orbits are closely set. Anterior border of orbit is a little projecting. Teeth are on both upper and lower jaws; of the upper jaw the larger. First dorsal fin originates above anterior third of the pectoral fin; the former is distinctly separated from second dorsal fin. First dorsal fin with six unbranched rays and second dorsal fin with one unbranched and ten branched rays. Dorsal fin rays are weak. Tip of first dorsal fin reach second dorsal fin origin and last ray of the latter reach caudal fin base. Pectoral fin, with 14- 15 rays, arises just behind opercle and it extends as far as below the middle of first dorsal fin. Ventral fin originate at the level of or a little in front of origin of pectoral fin. Its tip never reaches anal fin origin. Ventral fin with 1, 5 rays; fifth rays of each fin joined together its entire length to form a cup-like disk and not adherent to body. Anal fin with one unbranched and ten branched rays and is long based. It extends to caudal fin base. Dorsal profile of second dorsal fin and anal fin is straight. Caudal fin with 13 principal rays and is wedge shaped. Scales are quadrangular, soft and deciduous. Fifty scales present on lateral series and Head region is devoid of any scales.

Color: In life body is brownish yellow; dorsal, anal and caudal fins red or brownish red. 8-9 vertical bands present on sides which extends to back. Several irregular color bands scattered on head region. A conical dark band runs directly downwards from the eye to lower edge of preopercle. First dorsal fin has a black crescentic mark at its base bordered by curved white and black bands. A small black spot is present on the base of pectoral fin; another spot present under gill cover. After preservation in formalin second dorsal, anal and caudal fin becomes hyaline but distinct black spots become distinct on it.

Comparisons: Many researchers including TALWAR and JHINGRAN (1991) considered *Gobius malabaricus* as a s Synonym of *Stenogobius gymnopomus* (BLEEKER, 1853). WATSON (1991) synonymised it with *S. macropterus*. WATSON (1991) wrote: “This species may be synonymous with *Stenogobius malabaricus* (DAY 1865: 27), but until material can be examined from the type locality it cannot be placed there with certainty”. Major problem about ‘*Gobius malabaricus’* was the unavaibility of the reliable specimens of it in museums. Its type specimen (BMNH, 1889) was a dried one. It was impossible to evaluate diagnostic characteristics based on it.

The presently collected specimens were from its type locality (Karavannoor River of Trichur District) and its features coincide with the characters mentioned by Day (1865a, b); fin rays of dorsal, anal and caudal fin matching with DAY’S (1865) descriptions. Day wrote that its pectoral fin has 13 rays and ventral fin has 4 rays. It may be a printing mistake. All the fishes of this group have 1, 5 rays in ventral fin and the fifth one of both sides are fused together. Pectoral fin never has 13 fishes in these groups of fishes. In all other fishes he described along with this has 1, 5 rays in ventral fin and 17 rays (1, 16) in pectoral fin. In all other matters, as described earlier, Day’s description fully match with the characters of the presently collected fishes: fin rays except pectoral and ventral, lateral scale count, color of the body and fins, black spots on second dorsal fin, anal and caudal fin, first dorsal with a deep black crescentic mark, dark bar running downwards from the eye and irregularly disposed dusky vertical bands on the sides and back. (But the lateral vertical bands are more distinct in fresh condition. After preservation in formalin continuity of the bands were lost and seen as broken bands).

Fig. 1. *Gobius malabaricus*, ZSI/SRC F-9151; after Preservation in formalin

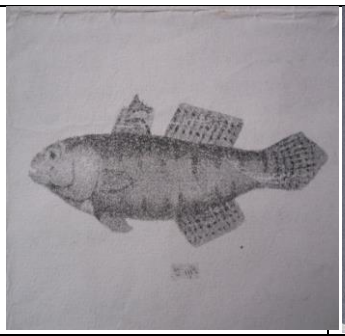

Fig. 2. Day's figure of *G. malabaricus*

Fig. 3. Fresh specimen of *G. malabaricus*

Fig. 4. Dorsal view of head

Fig. 5. Anterior region of *G. malabaricus*

Gobius malabaricus Day can be synonymsed with *Stenogobius macropterus* and not with *S. gymnopomus.* Charaters of *Gobius malabaricus* matches with *S. macropterus* than that of *S. gymnopomus*. In *S. gymnopomus* pectoral fin rays are 16 (vs. 14- 15 rays in *Stenogobius macropterus* and *Gobius malabaricus*) and first dorsal fin with brownish or blackish bars (vs. only crescentic mark below dorsal fin in *Stenogobius macropterus* and *Gobius malabaricus).* Moreover, type locality of *S. gymnopomus* is Priaman, Sumatra (vs. Ginganga near Vakvella, Ceylon of *S. macropterus*) far away from the type locality of *Gobius malabaricus* Day (Trichur, Kerala).

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EFFECT OF IMIDACLOPRID ON THE HAEMATOLOGY OF *APLOCHEILUS LINEATUS*

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ABSTRACT: The contamination of surface waters by pesticides used in agriculture is a problem of worldwide importance. Neonicotinoidsare the first new class of insecticides introduced in the last 50 years, and the neonicotinoid, Imidacloprid is currently the most widely used insecticide in the world. The present study was to estimate the effect and toxicity of Imidacloprid to non-target organism, the fish.*Aplocheilus lineatus*, a common freshwater fish in Kerala is highly sensitive to environmental pollutants and other anthropogenic disturbances.In the present study an attempt has been made to assess the suitability of this species in pollution bioassays and biomonitoring of environmental pollution. As the species is naturally available in aquatic habitats associated with agricultural fields, it is very convenient to study the impact of agricultural pesticides on fish fauna by assessing changes in their haematology.Results of the present study indicated that the widely used new generation pesticide, imidacloprid, especially at their higher concentration cause serious effects on the fish haematology. The pesticide induced an increase in the WBC count at 96 hr. exposure. Other haematological indices showed decreased values except the Hbcontent. Imidacloprid induced many significant morphological alterations in the blood cells.

Key words: Pesticides, Neonicotinoids, Imidacloprid, Haematology, *Aplocheilus lineatus*.

INTRODUCTION

Pesticides are one of the most potentially harmful chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are significant (JOHN, 2007; HAZARIKA and DAS, 1998). Due to injudicious and indiscriminate use of these agrochemicals such as fertilizers, pesticides, insecticides and fungicides to boost crop production with the sole aim of getting more yield, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted. Neonicotinoids are a class of neuro- active insecticides chemically similar to nicotine. The development of this class of insecticides began with work in the 1980s by Shell and the 1990s by Bayer. The neonicotinoids were developed in large part because they show reduced toxicity compared to previously used organophosphate and carbamate insecticides. Neonicotenoids include imidacloprid, acetamiprid, clothianidin, sulfoxaflor, nitenpyram, thiazine, thiacloprid, and thiamethoxan.The assessment of the eco toxicological risks caused by pesticides to ecosystems is based on toxicity data and the effects of pesticide preparations on non-target organisms. Fish are among the group of non-target aquatic organisms. SHANKAR MURTHY *et al*. (2013) published a research paper about the toxicity of pesticides in fish. Blood analysis is crucial in many fields of ichthyologic research and fish farming and in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes in fishery management and diseases investigation (ADEDEJI *et al.,* 2000). Physio-morphological changes in blood indicate the changes in the quality of the environment and therefore blood parameters are important in diagnosing the functional status of the fish exposed to toxicants (OKECHUKWU *et al.,* 2007).

A scan of literature in the field of pesticide pollution on fish fauna indicated that several researches have been carried out on the effects of organophosphorus, organochlorine and pyrethroids. However, effect of imidacloprid on haematological constituents has not been much studied. Very few studies are available on the toxic effect of imidacloprid on fishes. *Aplocheilus lineatus* is a common freshwater fish in Kerala inhabiting a wide variety of habitats such as pond, rivers, canals, wetlands etc. Very little studies have been conducted on its tolerance to various pollutants in the aquatic ecosystem. In the present study, the fishes are exposed to sub lethal concentrations of the pesticide in the laboratory for 96 hours to study the effect of Imidacloprid on fish haematology.

MATERIALS AND METHODS

Aplocheilus lineatus is a species of killifish belonging to the genus *Aplocheilus*. An aquarium variant of this species with a more yellowish colouration is known as golden wonder killifish. This species grows to a length of 10 cm (3.9 inch) TL.

Experimental design: Fresh water fish *Aplocheilus lineatus* were obtained from a local pond in Kollam district and were acclimatized under laboratory condition. They were kept in glass aquaria containing 20L of tap water. 24 fish were divided in 3 groups, 8 fish for each group: Group 1 served as control without any treatment of pesticide; Group 2 were treated with low dose of Imidacloprid i.e., 10 mg/L.; Group 3 were treated with high dose of Imidacloprid i.e., 20 mg/L.The fishes are exposed to different concentrations of the pesticide in tap water for 96 hours. The fishes were not fed throughout the duration of the sub-lethal toxicity tests.At each session, sixteen experimental and sixteen control fishes were selected at random and used for haematological examinations.

Haematological Estimations: Blood was collected from heart using heparinized disposable syringes containing 0.5 mg ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Indices measured included erythrocyte count (RBC), Haemoglobin concentration (Hb), haematocrit (PCV), mean corpuscular volume (MCV), mean cell haemoglobin concentration (MCHC), leucocyte count (LEU) and differential leucocyte count. The procedures were based on unified methods for haematological examination of fish (SVOBODOVA et *al.*, 1991).

Blood cell count: The Red Blood Corpuscles (RBC) and White Blood Corpuscles (WBC) were counted using haemocytometer crystalline chamber using “Hayem’s” and Turch’s” diluting fluid, respectively.

Haemoglobin Estimation (HB) and Packed Cell Volume (PCV): Haemoglobin was estimated by acid haematin method (SAHLI, 1962). Packed cell volume was estimated by Wintrobe’s tube (MUKHERJEE, 1988). These were analysed in SYSMEX Automated HaematologyAnalyser.

Mean Cell Haemoglobin Concentration (MCHC): This refers to the percentage of haemoglobin in 100 ml of red blood cell. This was calculated by dividing the haemoglobin content in g/dL by the PCV% of red blood according to the formulae: MCHC = HB/PCV ×1000 g/dL or HB × 10/RBC Count

Mean Corpuscular Volume (MCV): The value of the corpuscular volume was calculated from the haematocrit value (PCV %) and the erythrocyte count 10^6/µL) using the formula: MCV = PCV × 1000/RBCs fL or PCV×10/RBC Count

Mean Corpuscular Haemoglobin (MCH): Mean Corpuscular Haemoglobin concentration expresses the concentration of haemoglobin in unit volume of erythrocyte. It was calculated from thehaemoglobin value (HB) and from the erythrocyte count according to the formula**:** MCH =HB /RBCs pg or HB ×10 /RBC Count

Morphological examination of blood: Morphological examination of blood cells was done by preparing slides stained with Giemsa stain and photos were taken with help of research microscopes attached with camera at a magnification of 1000x.

Statistical analysis: Student's t-test was conducted for finding out the significance level of changes in haematological indices.

RESULTS AND DISCUSSION

In the present study, fresh water fish *Aplocheilus lineatus* was exposed to sub-lethal concentrations of Imidacloprid for 96 hours. For Imidacloprid the lower and higher doses of sub-lethal concentrations were 10mg/L and 20mg/L respectively. The fish show restlessness, rapid body movement, convulsions, difficulty in respiration, excess in mucus secretion, changes in colour, and loss of balance when exposed to the pesticide. Similar changes in behaviour are also observed in fishes exposed to different pesticides (HAIDER and INBARAY, 1986).

Some of the important blood indices showed significant variations in fishes exposed to the pesticide. Haematological indices, *viz*., RBC, WBC, HB, PCV, MCV, MCH and MCHC in the fish exposed to sublethal concentrations of Imidacloprid is given in Table 1 and depicted in Figures 1 and 2. The haematological analysis revealed a significant reduction in Red blood cell count (RBCs) from 0.09±0.010 $x10^6$/µL in the control to 0.08±0.015 $x10^6$/µL and 0.13±0.015$x10^6$/µLat low and high doses respectively. An increase in haemoglobin (Hb) was recorded from 0.40±0.100 g/dL in control to 0.40±0.265 µg/dL (low dose) and 0.87±0.503 µg/dL (high dose). Packed cell volume (PCV) increased from 0.70 ± 0.100 to0.57±0.057 and 1.06±0.208 at low and high doses respectively in fishes exposed to imidacloprid. There was a decrease noted in low dose for imidacloprid (0.57±0.057). The values of MCV showed a decrease from 78.05± 10.55 in the control to 67.09 ± 16.280 and 68.63 ± 18.337 in low dose and high dose respectively. The values of MCHC and MCH showed an increase in fishes exposed to imidacloprid. MCHC increased from 71.03±4.184 in control to 113.33 ± 5.075 and 116.98± 3.80 g/dL in low dose and high dose respectively. MCH increased from 55.64± 9.790 in control to 70.21 ± 24.468 in low dose and 91.45± 21.810 in high dose.Total WBC count showed a significant increase in fishes exposed to Imidacloprid at all concentrations. Total WBC showed an increase from 23.80 ± 0.200 in the control to 26.89 ± 0.250 and 68.84 ± 1.599 at low and high doses respectively in fishes exposed to Imidacloprid.

A significant reduction in RBC count was noted in low dose due to the exposure. An increase in RBC count was noted at high dose of Imidacloprid.WAHBI *et al.*, (2004) and ZAKI *et al.* (2008) attributed the decrease in the RBC to haemolytic crisis that results in severe anaemia in fish exposed to heavy metals and herbicide, respectively. Furthermore, the reduction of RBC also leads to development of hypoxic condition which in turn leads to increase in destruction of RBC or decrease in rate of formation of RBC due to non-availability of Hb content in cellular medium (CHEN *et al.*, 2004). Haemoglobin content showed a significant increase in fishes exposed to Imidacloprid. This result is in contradictory to many earlier works which reported a decrease in Hb content in fishes exposed to pesticides (MASUD and SINGH, 2013; RAMESH and

SARAVANAN, 2008). The present increase in the haemoglobin content is in correlation with erratic rise in the RBC values. The values of MCV in the experimental groups showed significant decrease at low dose and significant increase in high dose in the case of Imidacloprid.MCHC values showed increase at low dose and high dose. The MCV, MCH and MCHC values are completely dependent upon the factors of PCV, RBC count and haemoglobin concentration. In the present study, the PCV, RBC and haemoglobin concentration is completely altered. So indirectly the values of MCV, MCH, and MCHC were affected. In the present study the decreased PCV values with increased MCV and MCH associated with increased MCHC values could probably due to stress induced by the pesticides due to disturbances in haemopoietic activities of fish. Similar findings were also observed by a number of studies in different fishes (CHEN *et al*., 2004; MURAD *et al*., 1998., GARG *et al*., 1989; LAURENT *et al.,* 1999).

Table-1: Effect of Imidacloprid on haematological indices of *Aplocheiluslineatus*

Parameters	Control (0 mg/L)	Low Dose (10 mg/L)	High Dose (20 mg/L)
RBC 10^6 µl	0.09±0.010	0.08±0.015*	0.13±0.015***
HB g/dL	0.40±0.100	0.40±0.265**	0.87±0.503*
PCV %	0.70±0.100	0.57±0.057***	1.06±0.208***
MCV fl	78.05±10.55	67.09±16.280*	68.63±18.337*
MCHC g/dL	71.03±4.184	113.33±5.075***	116.98±3.80***
MCH pg	55.64±9.790	70.21±24.468*	91.45±21.810***
TotalWBC 10^3/ µl	23.80±0.200	26.89±0.250***	68.84±1.599***

***Significant p< 0.0001; **Significant p < 0.05; *not significant; ± S E

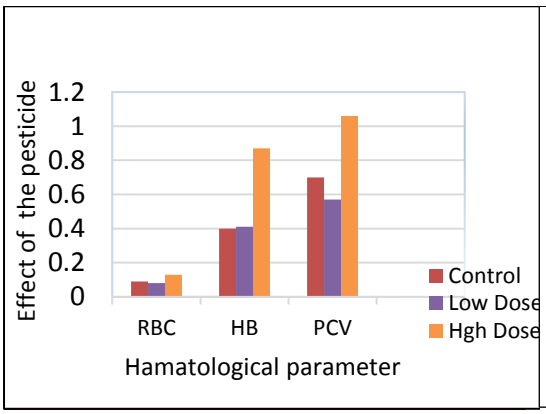

Fig.-1: Effect of Imidacloprid on RBC, HB and PCV of A. *lineatus*

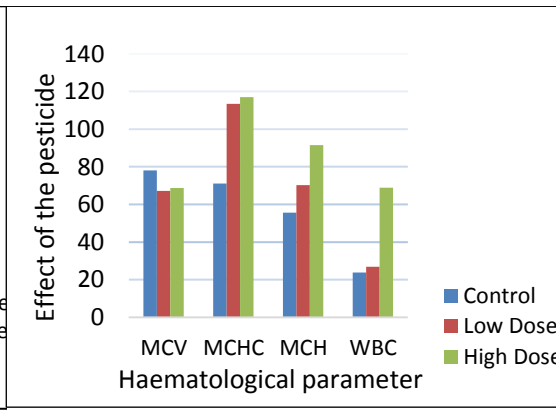

Fig.-2: Effect of Imidacloprid on MCV, MCHC, MCH and WBC of A. *lineatus*

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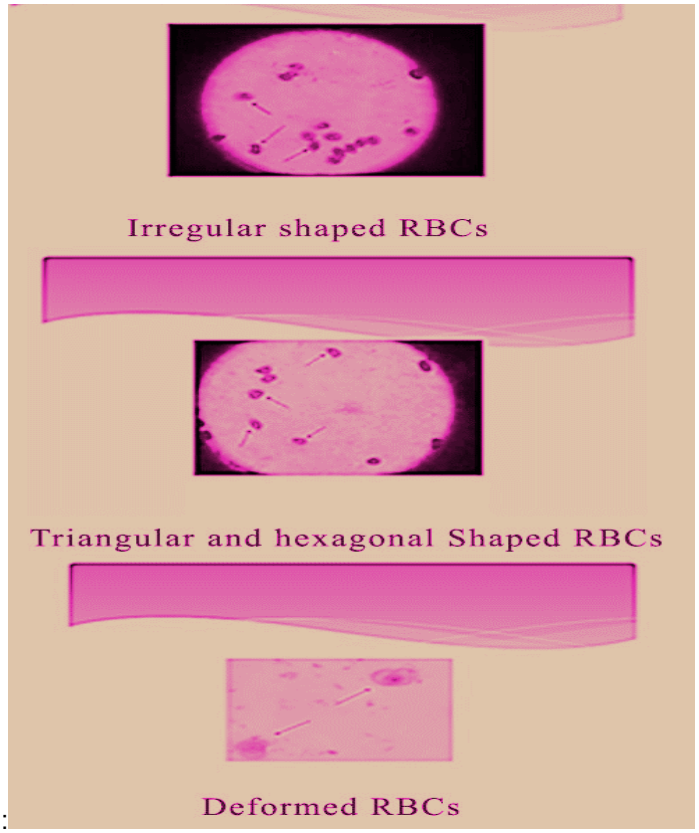

Morphological alterations in blood cells in fishes exposed to Imidacloprid
(Fig-3-5 above)

A dose dependent increase in WBC count was recorded in the present study in fishes exposed to the pesticide. WBCs are important cells in the immune system, because of their main defensive function. The WBC will respond immediately to the changing medium due to xenobiotic transformation. During exposure period of Imidacloprid the WBC counts got enhanced, indicating that the fish can develop a defense mechanism to overcome the toxic stress. The present study is in agreement with LOVELL and JANTRAROTAI (1991); NANDA (1997); WAHDI, (1998); HYMAVATHI and RAO, (2000); LEBELO *et al*. (2001); HASSEN, (2002) and JOSHI *et al*. (2002).

There were variations in blood cells exposed to Imidacloprid. Most common morphological alterations were in the change in the shape of RBC. Normal RBC of fish blood is oval in shape but in the present study the fish exposed to the pesticide showed RBC of triangular, hexagonal, irregular shaped and deformed RBCs. Such changes in the shape of RBC have also been reported by earlier workers (SAWHNI and JOHAL, 2000). RANJEET *et al*. (2013) has reported triangular and hexagonal shaped RBCs on exposed to Ekalux in *Anabas testudineus*. Fragmentation of RBC was also observed (JOHN *et al.,* 2004). These observations show that the pesticide have effect on the plasma membrane and also cytoskeleton which cause the alterations in shape. It can be concluded that though Imidacloprid is considered as possible replacement for the widely used organophosphate pesticides, however, at higher concentrations, Imidacloprid is toxic to

freshwater fishes similar to the toxicity of organophosphate pesticides at lower concentrations.

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CONTENTS

Contd. Contents at page 251