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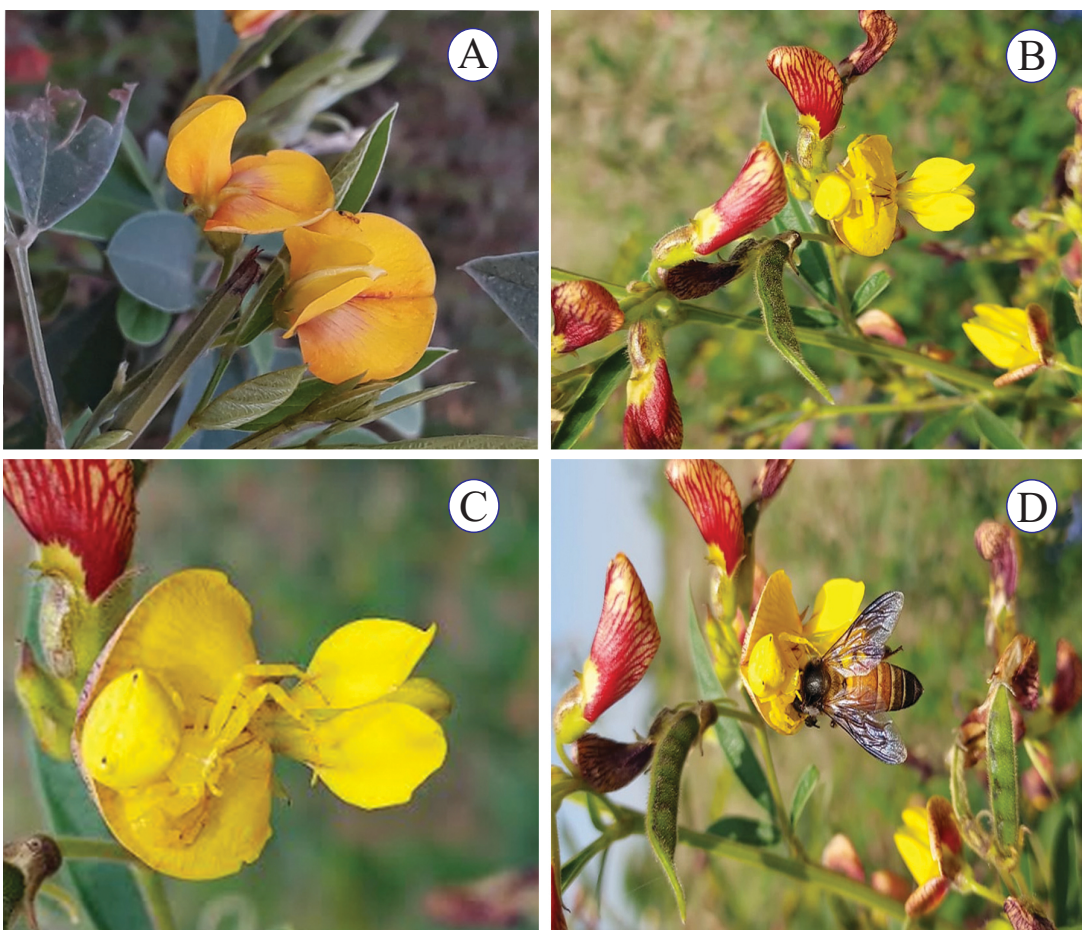

Fig. 1. *Thomisus onustus* mimicking the flower of *Cajanus cajan* for its predation; A. A typical papilionaceous corolla; B. *T. onustus* mimicking the corolla; C. *T. onustus* positioning itself under the vexillum of *C. cajan*. D. *T. onustus* ambushing the bee *Apis* sp. using the raptorial forelegs
For details see page No. 165 of this issue

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The main objective of the Society is to encourage and promote the dissemination of entomological knowledge. It arranges interactions of entomologists at the headquarters and at various places where the branches/ chapters of the Society are getting initiated. The annual general body meetings are held regularly and whenever necessary. These interactions provide opportunities to the members and others interested in the subject to keep in touch with the entomological activities, both in India and abroad. The Society has chapters, each with a minimum of 25 members and these conduct events for the promotion of Entomology.

The membership of the Society is open to all persons, above 18 years of age, who are interested in Entomology. Ordinary members have to pay an admission fee of Rs. 100/- and an annual subscription (pdf only) of Rs. 1000/-. From 2019, on payment of Rs. 15100/- one can have the option of straightaway becoming Life Member. Life membership had been restricted earlier to only those who have completed five years as ordinary members, but this condition has been waived off now, for some fixed duration. These payments are required to be made by online payment gateway at entosocindia.org or by NEFT transaction or in the form of DD or multicity cheque payable at New Delhi in favour of "Entomological Society of India". NEFT details are given in website. Life Members who wish to be considered as Fellows of the Society may send two copies of their biodata to the Entomological Society of India, Room No. 4A, Division of Entomology, IARI, New Delhi 110012. The biodata of the members will be screened by the competent committee of the Society before the declaration of the Fellows of the Society (FESI). The Society may occasionally elect Honorary Members, persons well known for their services to the cause of Entomology. For more details see website of ESI-www.entosocindia.org.

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The Indian Journal of Entomology: The official publication of the Society was started in 1939. Since 1956, it is being published as a quarterly journal and the four parts are published each in March, June, September and December. It is online published from 2008 (Volume 70) onwards (through indianjournal.com). It is open access w.e.f., 2022 Volume 84 and details can be seen in our ESI website and OJMS website indianentomology.org.

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The Journal of Grain Storage Research: Its single issue was brought out in April 2016. It was to be taken up further by the Society for Grain Storage Research, under the "indiastorageforum" which is still under formation. Any entity who/ which will like to take up this under the banner of the Entomological Society of India, may contact the Chief Editor.

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EDITORIAL

In the Editorial last year, I wrote about the "CHANGE" that is required to be pursued and the need for "changing" constantly. Yes, I reiterate that the measure of intelligence lies in the ability to "CHANGE". Such a change is imminent and is required to make Entomology purposeful and sensitive to the changing needs of science and society. This is especially so in serving its basic tenets and to make it strong and impactful. There is an imminent need to look into insects on a wider canvas and their diversity in a different mode and outlook so that insect communities, their functions and intricacies can be a forerunner for many understandings, and thereby benefit science and humanity. As had been repeatedly discoursed by me "Entomology provides multiple opportunities for researchers and scientists" in many domains. This is more so because of the multiplicity of the classes, species, populations, genes, ecology and habitats of insects and their communities. Because of these unique ramifications, insects offer numerous opportunities principally for exploring their intricacies in real time in both basic and applied fields. All of these provide valuable and potential clues for deciphering the science of biology in its real meaning especially when insects and life processes are used as models. Many of the life processes are getting explained and explored using insects and their organ systems as models, to provide the required strong scientific backing. Many happenings in biology are provided with strong and valuable explanations by resorting to deeply exploring insects and their organ systems. Many intriguing biological questions are explained with the concepts and hypotheses originating from insect models. Many things can be understood and learnt from insects and their life attributes. Naturally so, the learnings we derive from these are extraordinary and unique.

I quote here with pride such a unique explanation that emanates from a recent study on the brain of a fruit fly *Drosophila melanogaster* by few researchers from the Johns Hopkins University and the University of Cambridge. This study is giving far reaching conclusions of practical value in deciphering human brain and neural networks. Insect brains, upon the desired understanding, give us many clues about how the human brain works. In this study the researchers have explored fruit fly brain and came out with the "first ever mapping" of an insect brain. "Insect brain in many ways is similar to the human brain, and unravelling it will give us clues about the human brain and its working". This fact is all the more significant as there are only a few brain maps available today, and of the neurons making these. Neuron measuring roughly 10 billionth of a metre, and synapses junctions where neurons communicate with each other, need to be explored. It takes years to explore these and to reconstruct neural roadways, and of the synapses and neurons, despite deploying expensive equipment. But the results obtained with mapping of a fruit fly larva brain with 3,016 neurons has brought out path breaking results of significance in understanding the human brain. This has provided "a new way of breaking down brain structure into its simplest form, using the connections between neurons alone". Such a study using insect brain has led to developing "an algorithm that tracks signals across the brain" and it has been discovered that most neurons multitask, performing different roles depending on the particular sensory cues. Insects as models can thus pave the way for simplistic explorations of many biological pathways and there is a need to reinforce this principle in the minds of young and uprising entomologists. Many loose ends remain in insect biology because of the complexity of insects. There is need for emphasis to research such basic and fundamental aspects of Entomology. Let us machinate entomology and insects to appreciate this necessity and imminence, and strive to achieve this CHANGE.

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EVALUATION OF SILVER NANOPARTICLES GENOTOXICITY IN *HERMETIA ILLUCENS* USING COMET ASSAY

Eman A Abdelfattah

Entomology Department, Faculty of Science, Cairo University, Egypt
Email: eman.alaaeldein@gmail.com

ABSTRACT

Silver nanoparticles (AgNPs) are commonly used in various sectors such as food, cosmetics, medicine, and insect control. Otherwise, the toxicological effects of this promising technology should be studied to ensure its safety. This research aimed to investigate the potential toxicity of different concentration of AgNPs (0-20 mg/mL) on the cuticle cells of insects using alkaline comet assay. The level of DNA damaged was significantly higher in insects treated with 5-20 mg/mL AgNPs comparing to that from the control one with the fold of 1, 1.7, 1.9, 4.3, respectively in the tail length. A strong positive correlation occurred between concentration of AgNPs and all comet assay parameters were occurred with linear prediction equations. The possible deleterious impacts of AgNPs on the *Hermetia illucens* (L.) were discussed. Also, the potential using of comet assay as an accurate and cost-effective monitoring tool of fate of using nanoparticles was proposed.

Key words: Alkaline comet assay, DNA damage, biomarkers, silver nanoparticles, environmental fate, *Hermetia illucens*, oxidative stress, impact assessment, monitoring tool, cost-effective methodology, toxicity, nanoparticles safety

Nanoparticles are frequently used in a wide range of application. Nanoparticles are in high demand due to their unique physical and chemical properties as well as their ease to control. All these advantages are distinct from both free molecules and larger-sized particles. There are more than two million studies focused on the potential application of this promising technology (Yousef et al., 2019). Briefly, silver nanoparticles (AgNPs) are widely used such as an insecticide, a dye eliminator, an antifungal textile, and an antimicrobial or anticancer agent (Das et al., 2020; Xu et al., 2020). So, the potential genotoxicity of AgNPs contact application should be monitored. The ability of organisms to monitor the effect of releasing AgNPs into environment, the remediated ability of using fate, transport, or final disposal of nanoparticles, and the potential considered as environmental stressor were slightly studied (Ohore et al., 2021). Especially, insects are common in terrestrial ecosystems such as flies, and grasshoppers can be used as a sensitive assessment tool to stressor (Abdelfattah et al., 2017). In this context, environmental stress can increase the production of reactive oxygen species (ROS) in organisms (Abdelfattah et al., 2021). When ROS overload and exceed normal level, lead to oxidative stress causing deleterious effect to macromolecules of living organisms, including DNA damage, protein carbonylation, lipid peroxidation, and enzyme inactivation (Halliwell, 1999; Abdelfattah, 2016; Renault et al., 2016; Abdelfattah et al., 2017; Yousef et al., 2019; Abdelfattah, 2020; Nassar et al., 2020; Abdelfattah and Renault, 2021; Abdelfattah et al., 2021).

DNA damage may involve removing the bases that leads to strand breaks and consequently mutation (Abdelfattah et al., 2017). Single strand breaks of DNA damage can be measured using single cell gel electrophoresis (SCGE), or alkaline comet assay. This method is considered as one of the simplest, most sensitive and reliable method for detecting DNA strands breakages. The features of comet assay technique allow early detection of the stressor deleterious. Recently, the comet assay has become more common as a tool to study genotoxicity of various stressors in different animals, and in the last decade, also in insects (Abdelfattah et al., 2017). Hence, the aim of the study was to evaluate the damage level of DNA, using alkaline comet assay in the cuticle cells of black soldier fly (BSF) *Hermetia illucens* (L.), which exposed to different concentrations of AgNPs(l.) (0-20 mg/ mL).

MATERIALS AND METHODS

Polyvinylpyrrolidone (PVP)-coated AgNPs of mean sizes 20-30 nm was provided from Nanotech, Cairo, Egypt. Characterization of AgNPs was carried previously by Hafez and Fouad (2020). Besides that,

H. illucens was supplied from Entomology Department, Faculty of Science, Cairo University, Egypt. Before the experiment, the insect reared under the rearing condition (12:12 L: D; 34˚±2; 60% RH) in the cages size 30*30*40 cm for 100 adults. The feeding habits of adult are nectar feeding. So, AgNPs was applied in contact to adult by emersion the experimental cages 10*10*5 cm in different concentrations of AgNPs solutions (0, 5, 10, 15, 20 mg/ ml) for 48 hours post application. For each sub-group, 50 insects were dissected, after application, to isolate cuticle tissues for further analysis and were stored at −20 °C until use. Each experiment was done in three replicates.

The Single Cell Gel Electrophoresis assay (SCGE), known as the Comet assay was used to assess the DNA strand breaks according to Duroudier et al. (2021). The analysis of DNA damage was performed using OPTIKA B-350 fluorescent microscope (OPTIKA, Ponteranica, Italy), with a CCD camera. The image analysis system (Comet IV software) was used to quantify the single strand breaks of DNA by different parameters. Statistical analysis was performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp). A non-parametric test was carried out using the k independent Kruskal-Wallis test to compare between the effects of different concentration of AgNPs on comet parameters. Generalized estimating equation (GEE) was used to examine the effect of nanoparticles concentration on DNA damage. Reproduced and residual correlations between AgNPs concentration and all comet parameters of adult *H. illucens* were done based on Pearson's regression analysis.

RESULTS AND DISCUSSION

In the present study, the alkaline comet assay was used to evaluate the genotoxicity of promising nanoparticles contact as a recommendation of Souza et al. (2021) that include the fate of nanoparticles should be studied. *Hermetia illucens*, were treated with different concentration of AgNPs (0-20 mg/ ml) (Figs. 1 A-F, 2; Table 1). The contact effect of AgNPs can increase the production of ROS in the cells or tissues of living organisms and leads to oxidative stress (Ratan et al., 2020). The level of stress was evaluated in this study indirectly using oxidative damage of DNA in the cuticle tissue of *H. illucens*. This analysis was done according to previous accepted literature (Abdelfattah et al., 2017). The results revealed a significant increase of DNA damage in cuticle tissue of treated insect with different concentration of AgNPs compared to the control insects (Figs. 1, 2).

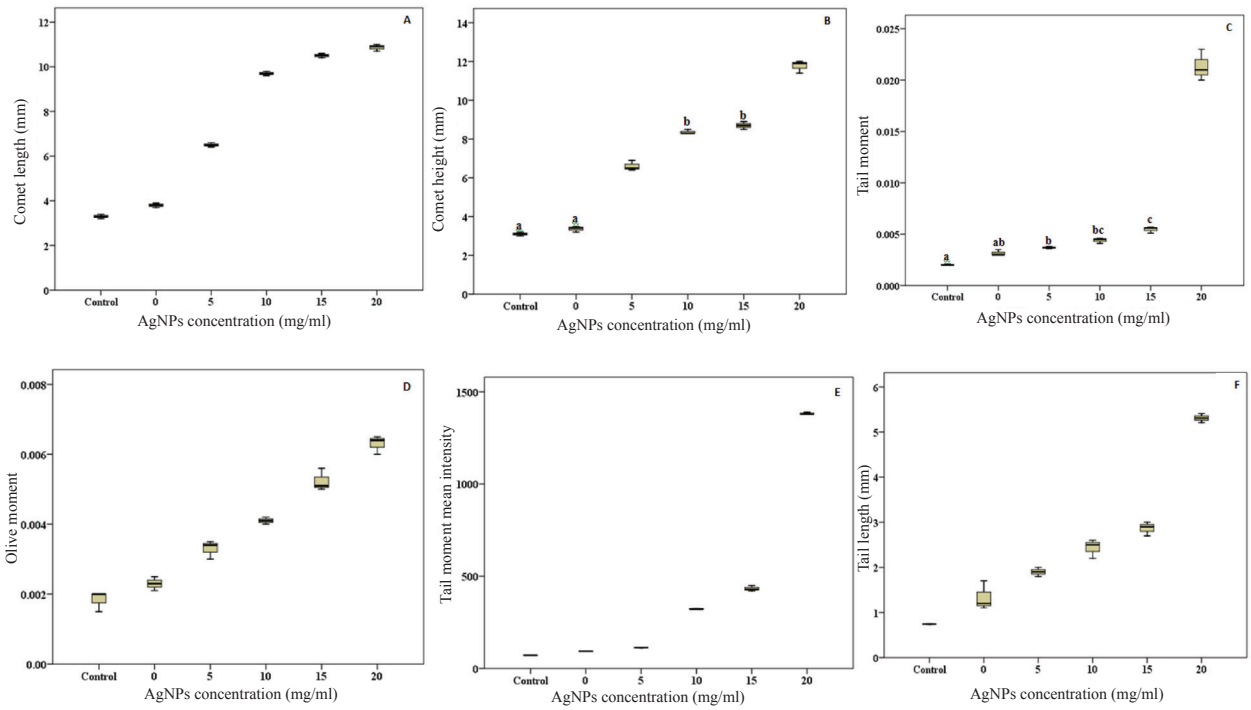

Fig. 1. Alkaline comet assay of cuticle cells males of *H. illucens* treated with AgNPs. Median values marked with different small letters significantly different (Kruskal-Wallis test, $p < 0.05$)

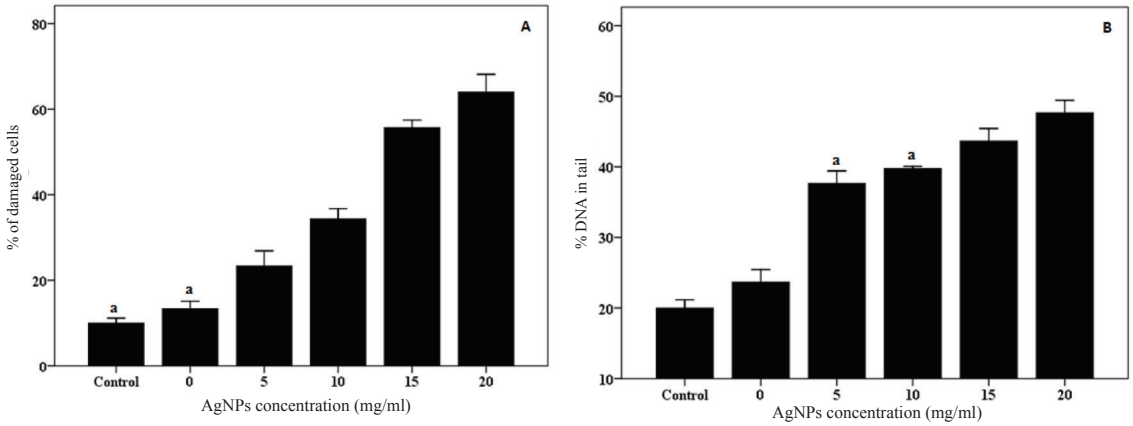

Fig. 2. DNA damage cells and DNA in tail % of cuticle cells males of *H. illucens* treated with AgNPs. Median values marked with different small letters significantly different (Kruskal-Wallis test, $p < 0.05$)

Table 1. Generalized estimating equation (GEE), regression equation, and correlation factor to analyze the interaction among concentration of AgNPs and intercept on comet parameters from cuticle of males *H. illucens*.

Item	QIC	Wald Chi-square	r	Estimated equation	R^2	*df*	P value
Concentration effect							
Comet length	12.14	22540	0.96	Y= 0.67X	-0.08	5	<0.0001
Comet Height	12.5	9156	0.97	Y= 0.60 X	0.24	5	<0.0001
Olive moment	12.1	1006	0.98	Y= 0.0005 X	0.69	5	<0.0001
Tail moment	12.2	2237	0.76	Y= 0.0008 X	0.60	5	<0.0001
Tail moment mean intensity	573.3	93402	0.83	Y= 50.5 X	0.71	5	<0.0001
% DNA in tail	32.7	2293	0.95	Y= 2.9 X	-0.29	5	<0.0001
% damaged cells	76.6	3700	0.97	Y= 3.4 X	0.85	5	<0.0001
Tail length	12.3	10968	0.92	Y= 0.24 X	0.66	5	<0.0001
Intercept							
Comet length	12.14	122427	-	-	-	1	<0.0001
Comet Height	12.5	30385	-	-	-	1	<0.0001
Olive moment	12.1	6356	-	-	-	1	<0.0001
Tail moment	12.2	2779	-	-	-	1	<0.0001
Tail moment mean intensity	573.3	93402	-	-	-	1	<0.0001
% DNA in tail	32.7	19532	-	-	-	1	<0.0001
% damaged cells	76.6	5604	-	-	-	1	<0.0001
Tail length	12.3	5034	-	-	-	1	<0.0001

The insect cuticle acts as the main and first site of defense. Also, it plays a key role in some essential activities (Ortiz-Urquiza and Keyhani, 2013). The various stressors can contact cuticle cells and lead to oxidative stress (Yousef et al., 2017; Abdelfattah and Renault, 2021; Abdelfattah et al., 2021). Investigations by Yasur and Rani (2015) showed differences in the activities of antioxidant and detoxifying enzymes, carboxylesterases (CarE), glucosidases (Glu) and glutathione S-transferases (GST) in the larval stage of lepidopteran gut after PVP-coated AgNPs treatment. Also, the activities of superoxide dismutase, catalase, and peroxidase were elevated in the larval bodies due to the AgNPs treatments. Besides that, the application of AgNPs at high concentrations led to induce heat shock protein 70, oxidative stress and apoptosis in *D. melanogaster* (Ahamed et al., 2010). These results agree with those of the present study which emphasized a strong positive correlation between AgNPs concentration and all comet parameters of treated insects also, a high level of significance of concentration and intercept effect of AgNPs treatment (Table 1). All these findings suggest

that nanoparticles exposure may induce oxidative stress, which can be indirectly detected through evaluation of macromolecules damage or the activity of antioxidant enzymes. The deleterious damage of DNA may occur as results of increasing levels of ROS. Also, in recent reports nano-Ag treatment led to damage the digestive system with symptoms of oozing of inner gut contents of lepidopteran larva, in addition to reduction the insect growth with prolonged larval period and larvae became sluggish, finally led to death.

Also, prolonged larval growth occurred in *S. litura* and *A. janata*, as a result of silver nanoparticles treatments was observed (Yasur and Rani, 2015). The present results corroborate with those of Lobo et al. (2010), who reported that DNA is considered as key target of free radical attack in the living cells. Abdelfattah et al. (2017) observed genotoxicity effect of different environmental stressors on different tissues of males and female grasshopper *Aiolopus thalassinus*. The relationship between comet parameters and different concentration of AgNPs treatment in the present study showed a unified pattern of a positive correlation. Asharani et al. (2008) revealed that AgNPs had a potential genotoxic effect and may cause chromosomal aberrations, DNA damage, and cell proliferation in cell lines of zebra fish. Nair and Choi (2011) focused on the exposure effect of different concentration of commercial silver nanoparticles (0.2, 0.5, and 1 mg/ l) on the aquatic midge *Chironomus riparius* (Meigen). The results emphasized the up and down expression of antioxidant enzyme glutathione-S-transferase (GST) genes as a result of nanoparticles application. Another study found that 4 mg/ l AgNPs concentration failed to show acute toxicity on *C. riparius,* These findings emphasize that the toxicity mechanisms of AgNPs depend on signaling transduction pathways which are associated with synthesis of proteins and activation of gonadotrophin releasing hormone; based on down-regulation of the ribosomal protein gene (CrL15) regulating ribosomal assembly, and upregulation of the gonadotrophin releasing hormone gene (CrGnRH1) or the Balbiani ring protein gene (CrBR2.2), respectively. Another study evaluated the effects of AgNPs on reproductive and pulmonary cells viability, lipid peroxidation, and total oxidative DNA damage. The results proved a strong cytotoxic activity of AgNPs at low concentrations (2-13 µg/ ml) and caused an overproduction of reactive oxygen species (ROS) (Zapór, 2016).

Many studies have evaluated the impact of AgNPs application, and these proved that the deleterious effect of AgNPs may occur as a result of direct cell membrane attachment, membrane integrity disruption, ROS generation, membrane permeability changes, proteins interaction, and DNA replication interference (Yu et al., 2013). Nanoparticles can bind to sulphur and phosphorus group and lead to degradation of proteins and DNA, respectively. So, the macromolecules damage occurred as a result of exoskeleton penetration by nanoparticles (Benelli, 2016). The present study concludes that contact treatment of AgNPs caused damaging effects on the DNA of *H. illucens* cuticle cells especially at highest concentration 20 mg/ ml as a result of ROS production. So, the genotoxicity study of AgNPzs- based products should be done before approval pf the products by decision makers.

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EVALUATION OF AN ODOUR DETERRENT TO BLUE BULL *BOSELAPHUS TRAGOCAMELUS* AND MONKEY *RHESUS MACAQUE* FROM AGRICULTURAL FIELDS

Ujjawal Kumar Singh Kushwaha

National Plant Breeding and Genetics Research Center,
Nepal Agricultural Research Council, Khumaltar, Lalitpur 00977, Nepal
Email: kushujjwal@gmail.com (corresponding author)

ABSTRACT

An experiment was conducted to evaluate two deterrents to deter blue bull *Boselaphus tragocamelus* and monkey *Rhesus macaque* from crop fields through randomized block design experimental trials in different parts of Nepal from July 2019 to May 2021. The odour deterrent tri methyl amine @15ml/ 700 m^2 and microbial fermented fish solution @4ml/ l were evaluated among 150 plots each with 50 replications including control. Significant results were obtained where >90% farmers responded that tri methyl amine repelled blue bull and monkey for >14 days. This might be due to its strong ammonia like fishy odour which was unpleasant to these animals and might had been found irritant and offensive. Similarly, many farmers replied that the microbial fermented fish solution also protected their crops from blue bull for ≥ 30 days.

Key words: Blue bull, *Boselaphus tragocamelus*, monkey, *Rhesus macaque*, wild boar, *Sus scrofa*, human-wildlife conflict, tri methylamine, odour deterrent, fermented fish

Nepalese farmers are severely afflicted with wild animals and the conflicts between them have been increased in recent days (Joshi et al., 2020; Paudel and Shrestha, 2018; Khanal et al., 2018; Thapa, 2016). The animal pests cause crop loss of million dollars each year and the damage may vary from 10-60% (Joshi et al., 2020; Pandey and Bajracharya, 2015). The extent of damage depends upon the cropping patterns, crop type, crop stage and cropping season (Schley et al., 2008; Cai et al., 2008). Because the protection of fields will remain essential in the future, thus it is important to address wildlife and human conflicts with appropriate techniques (Manral et al., 2016). Blue bull (*Boselaphus tragocamelus*) is a devastating pest of agriculture, mostly found in plain parts of southern Nepal (Koirala et al., 2020; Khanal et al., 2018; Thapa, 2016). They destroyed 14.48% and 10.38% of standing vegetables and pulses accounting $68,633 in Rupendehi district from March 2015 to March 2016 (Khanal et al., 2017). Blue bull could cause >50% crop damage in India (Meena et al., 2014). Physical barriers such as electric fences have been found promising, but their widespread uses are limited due to costs of construction, maintenance and no government subsidy (Hayward and Kerley, 2009; Pérez and Pacheco, 2006; Thapa, 2010).

Monkey is also a serious crop raider for hills and agricultural farms residing near the forests in plain parts of Nepal (Poudel and Shrestha, 2018; Sharma and Acharya, 2017). The annual crop damage by monkey was 183.46 kg ($75.10)/ household in Jaidi Baglung (Poudel and Shrestha, 2018) and >$267 economic loss was reported in Tallakodi Pokhara where maize (31%) was most raided crop followed by potato (30%) in 2015 (Sharma and Acharya, 2017). Similar to Nepal, the average economic loss was Rs.150 crore/ annum in Himachal state, India (Reddy and Chander, 2016) and 10-20% of total household income was lost each year in Rwanda near forest fragment area (Guinness and Taylor, 2014). Successful crop protection measures have not been identified yet except active guarding with domesticated dogs, beating with sticks and throwing stones over monkey (Bhatta and Joshi, 2020; Gehring, 2010; Uddin and Ashan, 2018).

Farmers use several strategies to protect their crops from devastating wild animals worldwide but none of them had provided complete protection because of varied nature of the animals and the taxa involved (Kolowski and Holekamp, 2006). Protective measures like modification in cropping patterns, cultivation of medicinal and ornamental plants are suggested to mitigate crop raiding (Manral et al., 2016; Guinness and Taylor, 2014). But they could cause significant reductions in food crops produced, thereby potentially increase food insecurity (Akankwasah, 2010; Vedeld et al., 2012). Blue bull, monkey and wild boar have widely developed olfactory organs. They use olfactation for orientation, foraging, intra specific social interactions and for avoidance of natural enemies (Schlageter and

Wackernagel, 2012). This background information reveals the fact that odour deterrent might be a promising means of deterring wild animal pests from agricultural fields. Thus, it was hypothesized that odour deterrent work against wild animal pests. In the present study, the efficacy of an odour deterrent chemical tri methyl amine (TMA) was investigated to deter free ranging blue bull, monkey, and wild boars from the crop fields.

MATERIALS AND METHODS

The experiment was conducted in randomized complete block design. Two treatments tri methyl amine (TMA) (Sun et al., 2018) and microbial fermented fish (MFF) solution were tested in 150 plots each with 50 replications in 2019-20 and 36 plots with 12 replications including control in 2020-21. A single farmer's field of ideal size 700 m^2 was considered as a plot and each plot worked as a replication. The control plots were selected where no deterrents placed and were >500 m far from the experimental plots. The study area consisted four districts of Nepal; Kathmandu (27.7172°N, 85.3240° E), Sindhupalchok (27.9512°N,85.6846°E), Sarlahi (26.9627° N, 85.5612°E) and Sunsari (26.6276°N, 87.1822° E). The study sites were chosen based on earlier complains by the farmers where blue bull, and monkey had high interference. The crops used were cabbage, cauliflower, potato, sweet potato, wheat, coriander, onion, chilli, brinjal, maize, pea, sugarcane, mustard, pigeon pea and okra.

A small bottle of size 60-100 ml was taken and about 12-15 ml TMA poured in it, and the cover of both bottles were made tight to prevent the spread of smells. The small bottle was placed in the center of the experimental field and a hole (0.1-0.2 mm dia) on the cover of the bottle was made to spread the odour slowly. The MFF was sprayed @4ml/liter of water. Four doses 5, 10, 15, and 20ml of TMA and four doses 3, 4, 5, and 6 ml of MFF solution were considered and distributed to farmers. Based on their perceptions, the dose was determined. There were no restrictions for the entry of wild animals in the experimental fields. Microbial fermented fish solution was prepared from helicopter catfish (*Wallago attu*) which was chosen because of its easy availability and fast degradability. About 1 kg fish was weighted and cut into pieces which were placed in a plastic bucket where 2 l of tap water and 200 ml of decomposer was also poured and mixed well. The container was covered and left for 70-80 days to ferment at room temperature (15-20^{o}C). When the fishes partially decomposed and started to emit odour at high level, it was sieved through cotton clothes in which 3 gm/ l fine chili powder was mixed, and again sieved and used as biorepellent. The decomposer used to enhance fish fermentation was claimed to contain natural microorganisms with trade name: EM-1 (Effective Microrganism-1), Balaju, Kathmandu, Nepal. The selected farmers were interviewed to know the status of wild pests in their farms before and after using the deterrents. Similarly, the estimation of damage of crops before and after using the deterrents were also reported and the collected data were subjected to construct ANOVA to know the level of significance for different variables through MSTAT software program. The number of wild pests visited in the experimental plots was considered as independent variables whereas the damages of crops were taken as dependent variables.

RESULTS AND DISCUSSION

The deterrent effects of tri methylamine and microbial fermented fish solution to deter blue bull and monkey from the crop fields were found highly significant compared with control (Table 1). The average crop damage before using TMA was 44% and it decreased to 3.6% with the use of deterrent. Similarly, crop damaged before using MFF was 34.9% and it reduced to 8.4% after spray. But the controlled trial crops were damaged maximum in 2019-20. In the same way, the crop damage before use of TMA and MFF solution were 35 and 38.75%, respectively and crop damaged after using the deterrents were 0 and 5%, respectively in 2020-21. The fields might be protected due to strong ammonia like fishy odour of TMA which was unpleasant to the blue bulls, and monkeys. The odour might had been found irritant and offensive and could had lost their orientation ability during field visit. But in control trials, blue bull ravages the fields with frequent trampling of crops causing severe loss. The techniques of using odour deterrents have been practiced since long time. Farmers use insecticides like phorate, phenyl solution, and thimet as deterrents which has strong fouling smell and could control blue bulls for a couple of days (Meena et al., 2014; Sitati and Walpole, 2006). Indigenous methods like use of audio/video shining tapes, scarecrows, beating of bells, live fencing, use of animal excreta, and fire crackers have been practiced widely but they do not work as expected (Bhatta and Joshi, 2020; Ansari, 2017). Similarly, etorphine hydrochloride, xylazine hydrochloride combined with ketamine is also used as chemical capture for blue bull (Tripathi and Rao, 2016).

TMA is a sensory pain causing deterrent which

Table 1. Effects of deterrents tri methyl amine and microbial fermented fish solution over animal pests- blue bull, monkey, and wild boar (2019-20, 2020-21)

SN	Treatments/ year	Animal pests in the field before using deterrents		Animal pests in the field after using deterrent		Farmer's crop damage before using deterrent (%)		Farmers crop damage after using deterrent (%)	
		2019/20	2020/21	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21
1	Tri-methyl amine	5	3	0	0	44.00	35.00	3.60	0
2	Microbial fermented fish	6	4	0	1	34.90	38.75	8.40	5
3	Control	5	4	5	5	43.10	34.58	34.60	55
Grand mean		5	3.78	2	1.889	40.66	36.11	15.53	20.13
Coefficient of variation (%)		45.46	40.15	93.46	89.19	47.33	41.98	123.49	41.88
Least significant difference at σ 0.05		0.98	1.25	0.65	1.39	7.63	12.57	7.61	6.99
p value		0.0135*	0.0433	0.0000*	0	0.0376*		0.0000*	0

*indicates significance; animal pests expressed in whole numbers by minimizing to decimals; crop damages expressed in %

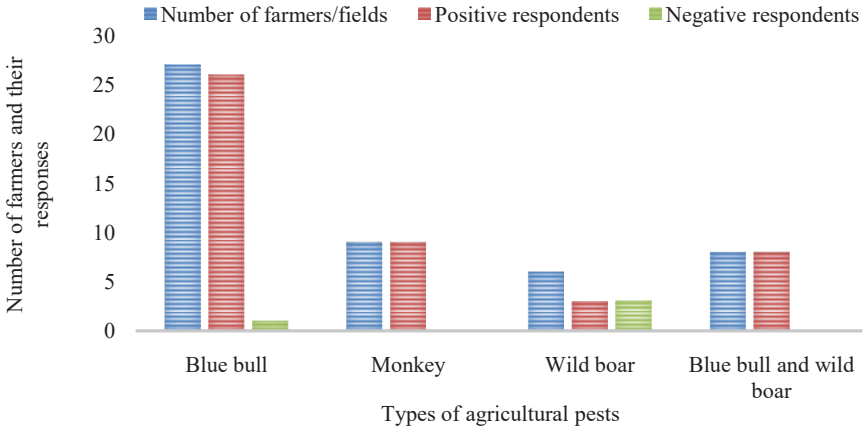

Fig. 1. Responses of farmers who used tri methyl amine. Positive respondents= wild animals leave the fields without damaging crops; negative respondents= wild animals damage crops (2019-20)

was found effective for blue bull and monkey but did not show adequate efficacy over wild boar. Farmers reported that wild boars did not respond exactly and continued to trample the fields where deterrents were placed. It meant one type of odour deterrent might not be effective for another type of wild species (Kolowski and Holekamp, 2006; Pandey and Bajracharya, 2015). Hence, still extensive trials are needed to confirm the results. An odour repellent "Wildschwein-Stopp" extensively tested to repel wild boars from the field was found ineffective (Schlageter and Wackernagel, 2012); but low dose warfarin baits proved effective to control pig problems (Poche et al., 2018). Farmers who used the odour deterrent for >two times at two weeks interval found that the blue bulls and monkeys got deterred for longer durations. Castor oil, egg solution, predator odours are also used for repelling blue bulls and monkeys but they are effective for shorter durations (Ansari, 2017; Meena et al., 2014; Tripathi and Rao, 2016; Parker and Osborn, 2006; Schlageter and Haag Wackernagel, 2011). Similarly, gonadotropin baits are seldom used for controlling monkeys (Tripathi and Rao, 2016). The deterrents causing pain are considered more effective than those causing fear or sickness, and thus chemical deterrents are widely used to protect a variety of crop species from wildlife damages (Schlageter and Wackernagel, 2012; Mason, 1997). Other methods based on acoustic, gustatory, and optic deterrence have not yielded satisfactory long-term results (Agyeman and Baidoo, 2019; Schlageter and Wackernagel, 2011; Sitati and Walpole, 2006).

Blue bulls did not damage the crops where MFF sprayed. Crops were safe and protected for about >30 days. The MFF solution had significant effects to deter blue bulls from the crop fields. MFF was also found equally effective to repel bulls and oxen. Since the MFF had strong fishy odour, it was disliked by herbivorous animals (Aryal et al., 2016; Meena et al., 2014). The effect of this biodeterrent was reported to be >5 weeks. Mixed responses about the effective durations of odour deterrent were obtained from the farmers who used TMA to deter blue bulls. The effective duration of odour deterrent varied but the duration was almost similar for monkey (Fig. 1). About 15ml TMA was found appropriate for ≥700 m^2 area to deter blue bulls

and monkeys for ≥12-14 days. The increase in amount of TMA indicated the voluminous spread of odour to the fields which was highly irritants to blue bull and monkey and reduced the chance of damage of crops and vegetables. Similarly, 4 ml MFF/ l of water was found more effective to repel blue bull for ≥30 days. Several other factors also determine the effectiveness of odour deterrent such as the target species, functionality of deterrent, time of placement, type of crop, and season etc. (Pandey and Bajracharya, 2015; Schlageter and Wackernagel, 2012). Tri methylamine is a sensory pain-causing chemical which was found highly effective to deter the wild animals during the experimentation. Therefore, this chemical compound can be used as a deterrent to avoid blue bull and monkey from the farmer's fields.

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CONFLICT OF INTEREST

The author declares no conflicts of interest.

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FORTUITOUS INTRODUCTION OF *ZYGOGRAMMA BICOLORATA* PALLISTER INTO BANGLADESH

R Muniappan* and Madhab Chandra Das[1]

CIRED, Virginia Tech, 526 Prices Fork Road, Blacksburg, Virginia 24061, USA
[1]Integrated Pest Management Activity, Virginia Tech, Dhaka, Bangladesh
*Email: rmuni@vt.edu (corresponding author): ORCID ID 0000-0002-2992-2792

ABSTRACT

The weed *Parthenium hysterophorus* L. was accidentally introduced to India in 1955 and from there it has spread to Bangladesh in 1988. A natural enemy of this weed, the leaf feeding beetle *Zygogramma bicolorata* Pallister was introduced to India in 1983 and it has fortuitously moved into Bangladesh. This communication reports on the first time finding of *Z. bicolorata* in Bangladesh on October 24, 2022.

Key words: *Parthenium hysterophorus*, *Zygogramma bicolorata*, biological control of a weed, leaf feeding beetle, fortuitous introduction, Bangladesh

The alien invasive weed parthenium *Parthenium hysterophorus* L. (Asteraceae) is of neotropical origin but has developed a pantropical distribution (Evans, 1997). It is now found in 92 countries around the globe, of which 44 are possibly in its native range (Shabbir et al., 2019a). Parthenium was introduced to India in 1955 (Rao, 1956), Nepal in 1967 (Mishra, 1991), Pakistan in the 1980s (Shabbir and Bajwa, 2006), Sri Lanka in 1987 and Bangladesh in 1988 (Pallewatta et al., 2003), and Bhutan in 1992 (Parker, 1992) (Fig. 1). It is one of the most damaging weed species present in natural systems and agroecosystems (Bajwa et al., 2016). It is an annual herb with a deep taproot system and it can grow up to two meters in height (McFadyen, 1992).

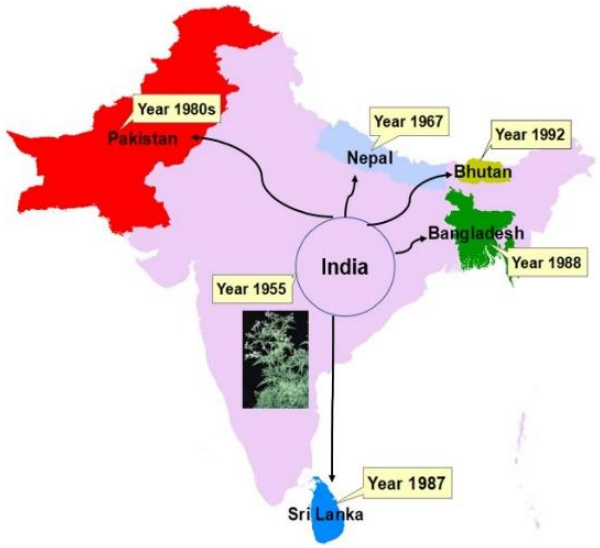

Fig. 1. Reported establishment of *P. hysterophorus* into South Asian countries

Management options include physical, cultural, chemical, and biological methods (Dhileepan and Strathie, 2009). Physical control involving slashing and pulling the weed can provide short-term relief but this type of control can cause health risks due to exposure to the weed. Management of parthenium with grass cover in Australia, and competitive replacement with *Senna uniflora* (Mill.) Irwin and Barneby, *Senna tora* (L.) Roxb. (Caesalpiniaceae) and *Tagetes erecta* L. (Asteraceae) in India have been reported (Shabbir et al., 2019b). Chemical control is expensive, as this weed grows in vacant lots, roadsides, parks, and recreation areas, and requires repeated applications. Classical biological control is one of the best options available for management of invasive species in general and parthenium in particular.

In Australia, biological control of parthenium was initiated in 1976 and since then 11 agents have been introduced for its management (Dhileepan et al., 2019). The leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), a native of Mexico, was imported into Australia in 1980 and field released in 1981. By natural spread as well as deliberate spread by farmers, the beetle has established though out Central, Southern, and Southeast Queensland (Dhileepan et al., 2019).

In India, biological control of parthenium was started in 1983, with the beetle being released in 1984 in Bangalore (Jayanth, 1987). The beetle has spread throughout the country (Viraktamath et al., 2004), and has fortuitously moved into neighboring countries. The

beetle was reported from the Punjab region in Pakistan in 2003 (Javaid and Shabbir, 2007). It has been collected, reared, and distributed to different parthenium-infested areas in the country (Rehman et al., 2017). The beetle was observed in 2009 for the first time in Hetauda at around 500 masl elevation of Nepal (Shrestha et al., 2010). It was then observed in other lowland areas of Nepal, such as Chitwan, Nawalparasi, Rupandehi in the same year. In 2010, the beetle was observed in Kathmandu at around 1200 masl. Its establishment was reported from Sri Lanka in 2019 (Pakeerathan, 2019) and Bhutan in 2020 (Dorji and Adkins, 2020) (Fig. 2).

Fig. 2. Reported establishment of *Z. bicolorata* into South Asian countries

On July 25, 2021, the USAID mission in Bangladesh awarded a cooperative agreement to Virginia Tech to manage current and emerging threats to agriculture in Bangladesh. Parthenium was identified as one of the weeds to be managed in the project. Current distribution of parthenium and the crops affected by it in Bangladesh have been documented (Karim and Ilias, 2022) (Fig. 3). Beginning in January 2022, the team has been conducting surveys in parthenium-infested areas along the roadsides and fields to detect possible migration of *Z. bicolorata* from India. The surveys were concentrated in the Khulna and Barisal divisions in the southwestern part of Bangladesh and failed to detect it. However, based on the report of establishment of *Z. bicolorata* at Malda district in West Bengal state of India (Dhileepan and Strathie, 2009) adjacent to Bholarhat upazila of Rajshahi division in Bangladesh, the authors conducted a survey on October 24, 2022 by examining parthenium along the roadsides and infested fields. At Chotto Jambaria village under Bholarhat upazila of Chapai

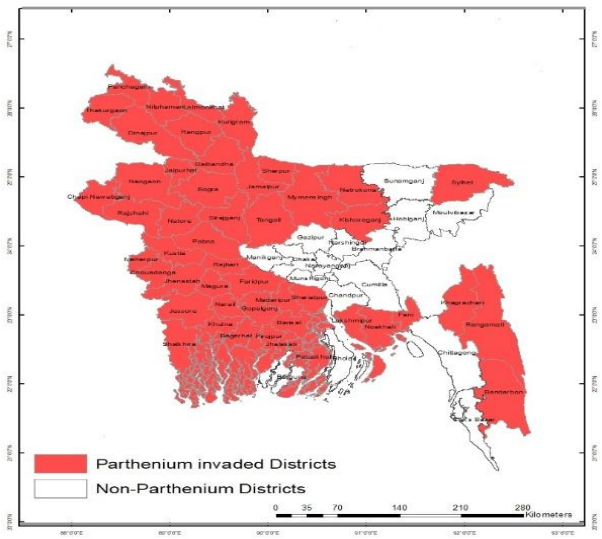

Fig. 3. Current distribution of *P. hysterophorus* in Bangladesh (Karim and Ilias, 2022)

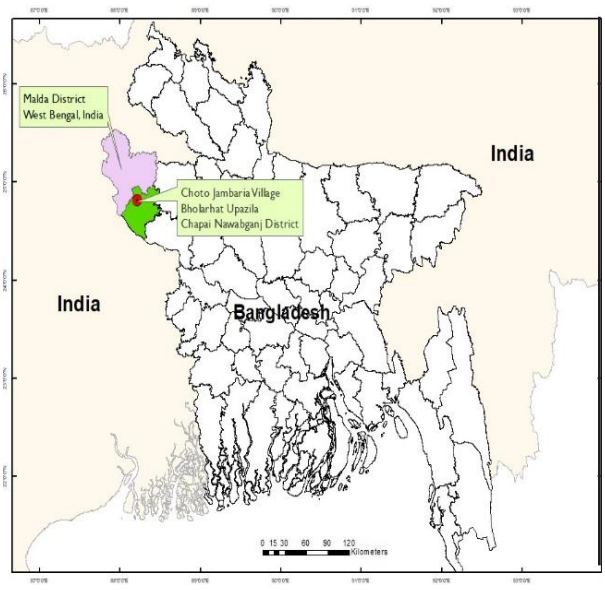

Fig. 4. Location of first finding of *Z. bicolorata* in Bangladesh

Nowabgonj district (24.8204471N, 88.2225504E), *Z. bicolorata* adults were observed on parthenium plants in a black gram *Vigna mungo* (Fabaceae) field. This is the first report of this natural enemy in Bangladesh and it is a fortuitous introduction (Fig. 4). Further surveys will be conducted in Bangladesh to delineate areas wherein *Z. bicolorata* has already established, conduct workshops to share knowledge with national stakeholders to develop an action plan, and rear and release this natural enemy in parthenium-infested areas throughout the country.

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AUTHORS CONTRIBUTION STATEMENT

RM conceived and MCD designed research. RM and MCD conducted field surveys. RM wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST/ COMPETING INTERESTS

Authors declare there is no conflict or competing interests.

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IDENTIFICATION OF MIRROR REPEATS WITHIN THE *MALELESS* (*MLE*) GENE OF *DROSOPHILA MELANOGASTER* MEIGEN

Saini Kavita, Dangi Namrata, Yadav Deepti and Bhardwaj Vikash*

Department of Zoology, Baba Mastnath University, Asthal Bohar Rohtak 124021, Haryana, India
*Email: vikashbhardwaj@gmail.com (corresponding author): ORCID ID 0000-0001-6569-665X

ABSTRACT

DNA repeats present in prokaryotes and eukaryotes genomes that include simple tandem repeats, satellite DNA, or palindromic sequences are classified as inverted, direct, and mirror repeats (MRs). Out of these, MRs are not well studied in *Drosophila melanogaster*. In this study, manual bioinformatics approach was used to find MRs in *D. melanogaster* maleless *(mle*) gene. In this analysis, 123 MRs were found within the complete *mle* gene, while 78 MRs were found in the exonic region of the *mle* gene. MegaBLAST was performed to elucidate the presence of identified MRs across the genomes of *D. melanogaster, D. nasuta* and *D. bipectinate*. These demonstrated the conserved characteristics of specific MRs in *Drosophila* genome and the evolutionary and functional significance of MRs in diverse genomes. This study establishes a link between the presence of MRs in *mle* gene of *D. melanogaster* and MRs in human genome.

Key words: DNA repeats, palindromic/ gene sequence, inverted/ direct repeat, mega BLAST, repetitive DNA, perfect/ imperfect mirror repeats, parallel complement, sex determination gene, exons

The DNA molecule serves as a central repository for genetic data. Genetic data held within the DNA regulates a wide range of biological functions. In addition to its normal B-DNA form, DNA can acquire a variety of shapes as a result of mutations, strand breakage, and inadequate crossing over. This can result in the formation of repetitive sequences within the genome (Gurusaran et al., 2013; Jain et al., 2008; Zattera et al., 2020). Any organism possesses homologous DNA segments in multiple copies, which are referred to as repetitive DNA sequences, which can be essential for regulating the cell cycle, gene expression, chromosome structure, and karyotypic evolution (de Koning et al., 2011; Jurka et al., 2007; Mehrotra and Goyal, 2014; Zattera et al., 2020). These repeats impact important biological processes such as translation, transcription, recombination, and chromatin structure formation. Moreover, the formation of H and Z DNA by short repetitive sequences can be intrinsically mutagenic in bacteria, yeast, mouse and mammalian cells (Pandya et al., 2021; Wang and Vasquez, 2017). The small repetitive sequences on the basis of the alignment of nucleotide sequences can be classified as direct, inverted, and mirror repeats (Gurusaran et al., 2013; Jiang et al., 2018; Mirkin, 2001). When a repeating DNA fragment is inverted on the same strand, it is called an "inverted repeat" (Mirkin, 2001). They play an important role in the regulation of transcription and translation (Varshney et al., 2020). Even in bacteria, inverted repeats and their associated hairpin structure are frequently found as a part of the independent transcription terminal (Brazda et al., 2020; Lillo and Spanò, 2007). Direct repeats are the sequences that are repeated multiple times on the same strand of DNA in the same orientation (Mirkin, 2001). Both direct and inverted repeats can stimulate the genetic rearrangements that cause deletion and duplication of genetic material (Lovett, 2004; Marie and Symington, 2022).

On the other hand, repetitive DNA sequences are divided into two groups based on their distribution within the genome: tandem repeats and interspersed repeats (Jurka et al., 2007; Pathak and Ali, 2012; Zattera et al., 2020). Long interspersed elements (LINES) and short interspersed elements (SINES) are two forms of interspersed repeats based on the length of DNA sequences, while microsatellite, minisatellite, and satellite DNA are types of tandem repeats (Mehrotra and Goyal, 2014; Pathak and Ali, 2012; Zattera et al., 2020). Mirror DNA repeats are small repeat sequences with bilateral or central symmetry in the same strand (Mirkin, 2001). It means that one section of the sequence fragment is an exact replica of another. In the case of mirror repeats (MR), parallel complement and anti-parallel complement are the same, while in normal DNA, parallel complement and anti-parallel complement are not identical.

The model organism *D. melanogaster* is employed

for the study of numerous human disorders because it contains distinct genes that regulate various developmental processes. Numerous homologous genes are known to play a role in human development and disease (Mirzoyan et al., 2019; Perveen, 2018; Tolwinski, 2017). *Drosophila* is useful for studying several human diseases such as neurodegenerative disorders viz., Huntington's, spinocerebellar ataxia and Alzheimer's disease (Bolus et al., 2020; Ugur et al., 2016). The mechanisms of determining *Drosophila's* sexual differentiation have been revealed through genetic, developmental and molecular investigations (Chen et al., 2019; Cugusi et al., 2015; Gadagkar et al., 1982; Oliver et al., 1993) The ratio of X chromosomes to sets of autosomes (X:A), which is a chromosomal signal, was used to control a small number of regulatory genes, which in turn direct differentiation to produce the morphological, physiological, and behavioural distinctions that differentiate males and females (Nöthiger and Steinmann-Zwicky, 1987). Maleless (*mle*) is required in both somatic and germ cells for male *D. melanogaster*. *Mle* is essential for X-chromosome dosage compensation in somatic cells (Lv et al., 2019). Unknown is the function of *mle* in the germline (Cugusi et al., 2015; Rastelli and Kuroda, 1998). The current study aims to identify the mirror repeats in the *D. melanogaster* maleless (*mle*) sex determination gene as well as to study the wide distribution of identified mirror repeats within the whole genome of *D. melanogaster* and other related species of *Drosophila*.

MATERIALS AND METHODS

The nucleotide sequence of the *mle* (maleless) gene of *D. melanogaster* (gene Id- 35523) was retrieved from NCBI (National Centre for Biotechnology Information) in FASTA format. The 5922-base pair long *mle* gene was fragmented into 500 base pair segments. The resultant fragmented sequences were treated as query sequences, and the reverse complement of the coding sequence was generated by a reverse complement tool (https://www.bioinformatics.org/sms/revcomp.html) and treated as the subject sequence. Both the sequences were aligned for similarity in the local regions using the BLAST tool. Fig. 1 illustrates the strategy used to identify mirror repeats. Using the strategy, the search was also conducted for mirror repeats within the break point region (the point where it was fragmented out of the gene into 500 nucleotide base pairs). https://nonb-abcc.ncifcrf.gov/apps/nBMST/default/ Non-B DNA Motif Search Tool was used to search for mirror repeats within the *mle* gene.

The programme parameters were fixed, word size 7 was used in the alignment and hits were observed at different expected threshold values (E-values). The E-value at which the greatest number of hits was observed was used to identify mirror repeats. Mirror repeats were identified in alignments where the position number in the subject and query sequences was the same in reverse order. The mirror repeats were classified based on the presence of a spacer between the

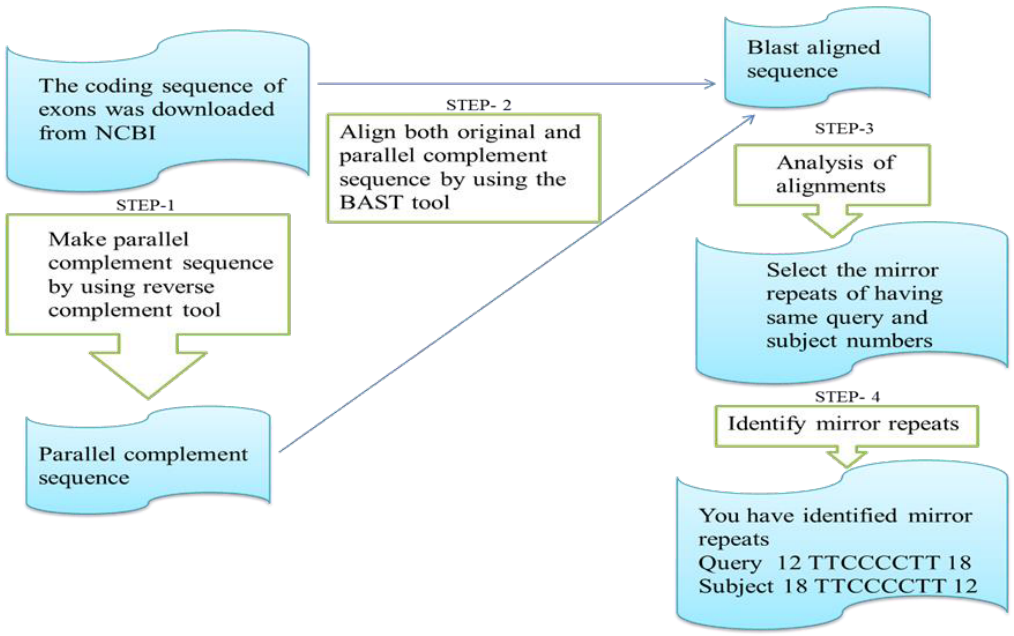

Fig. 1. Schematic representation of the methodology used to identify mirror

subject and query sequences. Here, Mega BLAST was used to search for the identified mirror repeats in the entire genome. Eventually, a phylogenetic analysis was performed to determine the conserved characteristics of these mirror repeats in different genomes like *D. nasuta* and *D. bipectinate*. During the search, algorithm parameters including word size, expected threshold, and maximum target sequence were adjusted to maximize the hits.

RESULTS AND DISCUSSION

In this study, a manual bioinformatics approach was used to find the mirror repeats within the *D. melanogaster's* maleless *mle* gene, a sex determination gene in *Drosophila*. A straightforward bioinformatics approach was employed to locate mirror repeats within the *mle* gene of *Drosophila melanogaster,* which is involved in the sex determination of *Drosophila*. The total nucleotide sequence length of *mle* gene is 5922 nucleotide base pairs with 6 exons. 123 mirror repeats were identified in the *maleless* gene (*mle*) at an expected threshold (E-value) 20. Of those 78 mirror repeats were dispersed between the six exons of the *mle* gene. As the *mle* gene (taking 500 nucleotide base pairs) was divided into 12 segments, a total 11 breakpoint regions were created. Moreover, mirror repeats were also searched within each break point region. Out of 11 breakpoint regions, only one breakpoint region was able to detect mirror repeats. Consequently, in the mle gene, 124 mirror repeats were identified. The repeats known as "perfect mirror repeats" are those that have identical sequences aligned around a central axis. A perfect mirror repeats may have a spacer element in between them, which was termed a perfect mirror with one spacer. Mismatches can be seen in imperfect mirror repeats around the central axis. The occurrence of the perfect with one spacer mirror repeat type was present more in all fragmented sequences, as shown in Table 1. In the maleless gene, out of 124 mirror repeats, 110 were perfect mirror repeats and 14 were imperfect repeats.

In the *mle* gene, 88% of identified mirror repeats are perfect mirror repeats, as shown in Fig. 2. In the fragment 1501-2000 of the *mle* gene, the maximum number of mirror repeats was observed (19 with one perfect, six imperfect, and twelve perfects with one spacer type). Thus, this region within the *mle* gene is rich in mirror repeats. However, the minimum number of mirror repeats of 5 was found in the region of 5001–5500 with five perfect with one spacer type in the *mle* gene, as shown in Table 1. The composition of perfect mirror repeats (P) and imperfect mirror repeats (IP) in complete genes and in exons is shown in Fig. 2.

Table 1. Mirror repeats in *mle* gene and their types at the expected threshold E-value 20

CDS	Expected threshold	Hits	Mirror repeats	Types of mirror repeats
1-500	20	46	14	P- 4 IP-1 POS-9
501-1000	20	30	10	P-1 IP-3 POS-6
1001-1500	20	28	10	IP-1 POS-09
15001- 2000	20	65	19	P-1 IP-6 POS-12
2001-2500	20	23	09	P-2 IP-2 POS-05
2501-3000	20	21	09	IP-1 POS-08
3001-3500	20	48	12	P-04 POS-08
3501-4000	20	42	08	P-01 POS-07
4001-4500	20	28	08	P-03 POS-05
4501-5000	20	18	06	POS-06
5001-5500	20	24	05	POS-05
5501-5922	20	55	13	P-04 POS-09

P stands for perfect, IP stands for imperfect and POS stands for perfect with one spacer mirror repeats

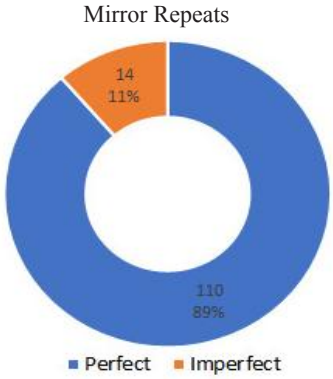

Fig. 2. Composition of perfect mirror repeats (P) and imperfect mirror repeats (IP) in (a) complete *mle* gene of *D melanogaster*

To further investigate the distribution of identified mirror repeats across the species, Mega BLAST tool was used. It was observed that the distribution of 25 mirror repeats has a length of 10+ base pairs by using

the Mega BLAST tool. As a result of the Mega BLAST tool's restrictions, which include a word size limit of 16, it was unable to detect all small-scale mirror repeats. Table 2 contains the entire Mega BLAST result for the large mirror repeat found in various parts of the mle gene. Out of 25 repeats, two repeat sequences were not found in the genome of *D. melanogaster*, which clearly indicates there is a need to upgrade BLAST/ Mega BLAST tools for efficient search for small nucleotide sequences. The ancillary file contains the entire Mega BLAST result for all mirror repeats. Lastly, non-B DNA motif search tool (nBMST) repeats were used within the mle gene. Only one mirror repeat "TATTGGAGGAGGATATGGAA ATAATGCAGGAGGTTAT" having a 17 nucleotide base pair spacer was found using this tool, the result of non-B DNA search tool is shown in Fig. 3. It was observed that there is a need to develop bioinformatics tools that can identify mirror repeats in a gene, clearly and quickly.

Using a simple manual computational approach, it was possible to detect 124 mirror repeats within the mle gene of *D. melanogaster*. This study can contribute to the development of new tools in molecular and bioinformatics approaches that can help elucidate the mirror repeats' genomic relevance. This kind of study has not been done before. Furthermore, molecular and biophysical research could determine how to comprehend the molecular function of identified mirror repeats.

Sequence_name	Source	Type	Start	Stop	Length	Score	Strand	Repeat	Spacer	Permutations	Subset
seq	ABCC	Mirror_Repeat	5370	5406	37	NA	+	10	17	1	0

Composition	Sequence
3A/0C/4G/3T	tattggaggaggatatggaaataatgcaggaggttat

Fig. 3. Result of non-B DNA motif search tool

Table 2. Distribution of selected mirror repeats (10+ bp)

Identified mirror repeats in *mle* gene of *D. melanogaster*	*D. melanogaster* (genome)	*D. nasuta* (genome)	*D. bipectinata* (genome)
ACCTATATTGGTGTG GGTAATTCCA	+	-	-
ACAAGCAGACGAA CA	-	-	-
GAACATTTACAAG	+	-	+
GCTTCCTTTTCAACTTT–CTTCG	+	-	-
TGTCGGGCTGT	+	-	+
ATGGCGGTGACGGC GGTA	+	-	-
CGCCGGCAGAACGA—-TGTGGTACGAT GCTCCGAACTACA	+	-	-
—AGAACCACGTCC AGCTTCCTTGCGTG GGGTCTGTAAGCAA AACGGACGC			
TCTGTCGGCTGGCT	+	-	-
TTATCAGACAAAGA CTATT	+	-	-
TATATTATTATAT	+	-	+
TTTAGAGATTT	+	-	+
CTAAAATCTGCGTCTTTAATC	+	-	-
TTAGCTAAATGTTG TATACCGGTT	+	-	-
CGAAACAACAATGC	+	+	+
TACTTTATAAA	+	-	+
TCTATATATAGCT TTAAATT	+	-	-
AAAATGTAGTGTAC GATGTAAAA	+	-	-
GGGCTCCTCCTCAG G	+	-	+
TAAGCTCGAAT	+	-	+
GTGGCCGAGCGCGTAGCT-CGCGAG-CGG TG	-	-	-
CATTAAATTAC	+	-	+
CTTGATATAGGTC	+	-	-
AAAAACAAAAA	+	+	+
ATACATACATA	+	+	+
TACATACATATAT	+	+	+

+ denotes presence and – denotes absence

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AUTHOR CONTRIBUTION STATEMENT

Vikash Bhardwaj: Conceptualization, methodology, software, supervision, reviewing, and editing; Kavita Saini: Data curation, investigation, and writing-original draft preparation; and Namrata Dangi: Writing- review and editing.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest.

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CHARACTERISATION OF THE GUT BACTERIOME OF HILL AND PLAIN RACE OF INDIAN HONEY BEE *APIS CERANA* FABRICIUS

Akanksha Thakur, S Mohan Kumar*, N Saranya, S Nakkeeran[1], M R Srinivasan[2] and S Subramanian[3]

Centre for Plant Molecular Biology and Biotechnology,
Tamil Nadu Agricultural University (TNAU), Coimbatore 641003, Tamil Nadu, India
[1]Agricultural College and Research Institute, Kudumiyanmalai, Pudukkottai, TNAU
Coimbatore 641003, Tamil Nadu, India
[2]Department of Entomology, Centre for Plant Protection Studies, TNAU
Coimbatore 641003, Tamil Nadu, India
[3]NBB - Gut Microbiome Project Consortium, Division of Entomology, ICAR- IARI, New Delhi, India
*Email: mohankumar.s@tnau.ac.in (corresponding author) ORCID ID: 0000-0002-1581-7318

ABSTRACT

Microbial communities are virtually present in every site of the body of the animals, but the ones associated with the gastrointestinal tract are home to a vast majority of microorganisms and are of importance due to their diverse impacts on animal health. The guts of the honey bees also consist of a distinctive microbiome and traditionally culturing technique was used for the exploration of their gut bacteriome. However, this did not give us a complete picture of the bacterial community. Recently, a more complete and precise picture of the potential bacteria can be explored using next-generation sequencing. The honey bee gut bacteriome is an essential aspect of bee health and this study is aimed at the microbiome of the bee gut by targeting the V1-V9 hyper variable region of the 16S rRNA gene with Nanopore sequencing using adult worker bees was performed from the plain and hill regions of Coimbatore to understand the bacteriome variations. A total of 3, 88,947 reads were obtained revealing five phyla and the gut of the bees was found to be dominated by the Proteobacteria. In addition to the metagenomics approach, the traditional method of isolating bacterial species using the culturing techniques was also done and a BLAST search was performed for the identification of the cultural isolates using the universal bacterial primers. The number of unique OTUs for hill and plain races were 30 and 9, respectively, and 9 OTUs were common to both the races. In hill race, Actinobacteria was unique and in plain race, Bacteriodota abundance was more. Bacteriome profile variation was also observed in plain and hill honey bee races mostly at the species and genus level.

Key words: Indian honey bees, *Apis* spp. colour morphs, gut bacteriome, 16S rRNA gene, V1-V9 metagenomic sequencing, Operational Taxonomical Units (OTUs),

In India, crop production is an important aspect of the economic sector with agricultural yield contributing significantly to achieve food security, reducing the poverty in the country, the generation of employment, economic growth and environmental sustainability. Honey bees are the primary source of pollination (Klein et al., 2007) and majority of the food consumption for humans is dependent on bee pollination. Honey bees, the flagship pollinator species, are social insects (Gilliam, 1997) and live in a community (Miroslava, 2019). The taxonomic classification of honey bees is Arthropod phylum, Insecta class, order Hymenoptera and family Apidae. Honey bees yield honey and a number of other by-products including beeswax, propolis, royal jelly and honeycomb.

In Tamil Nadu, four honey bee species of *Apis* genera are well recognized. These include-*Apis cerana* (the Indian honey bee); *Apis dorsata* (the giant honey bee); *Apis florea* (the dwarf honey bee); and *Apis mellifera* (the western honey bee). Besides, many species of stingless bees are also utilized for gathering honey on a smaller scale. Among these, *A. cerana* is economically reared to get honey and other products. *A. cerana* is found at varying altitudes with appropriate flora and climate. In South India, based on morphological features, two "races" of *A. cerana* are identified: a black 'Hill' morph, that is often said to live at a higher elevation and a yellow "plain" morph found at lower elevations (Shashidhar et al., 2013; Fakrudin et al., 2013). The black and yellow morphs of *A. cerana* are reported from Kerala, Karnataka, Tamil Nadu and Andhra Pradesh (Shashidhar et al., 2013). The population from Karnataka also showed closer

relatedness with the bees from Assam and Jammu. Honey bees from mountainous zones were darker in color than those collected from sub mountainous zone. It was reported that the bees from higher altitudes and cold temperate regions were markedly darker than bees from lower altitudes and warm subtropical regions. Various studies confirmed that the morphometric features of *A. cerana* honey bees showed a wide range of variation in measurements of size, and colour pattern but they are not reproductively isolated/ genetically distinct (Shashidhar et al., 2013; Fakrudin et al., 2013).

The importance of gut-dwelling microbial communities in the health of animals, from humans to insects, has become widely appreciated (Moran, 2015). Honey bees also share a diverse microbiome with different bacterial taxa, ranging from gram-positive bacteria to alpha-, beta-, and gamma-proteobacteria (Gilliam, 1997; Jeyaprakash et al., 2003). Initially studying the microbes was possible only in laboratory environments, where the traditional culture-based methods gave an incomplete picture of the microbial communities. Recently the non-laboratory techniques have now become much more powerful and these approaches based on DNA sequencing have enabled more reliable results. The new methods have revolutionized our understanding of microbial ecology and provide insights on the dynamics of the communities and their potential effects to the organism (Moran, 2015; Zheng et al., 2018).

The 16S rRNA sequence has been immensely exploited to distinguish the strains based on the polymorphism within the gene. Recently, with the availability of the next-generation sequencing approaches, full-length gene sequencing is possible. Although the hyper variable region V3-V4 of the genome is widely being sequenced, the full-length gene sequencing from the first to the last hyper variable region would provide better coverage of more bacterial populations (Johnson et al., 2019). Although representative genomes of *A. cerana* micro-biota have been sequenced, considerable variation between strains, indicating the existence of large pan genomes within each microbial species that encode a diversity of genes and functionalities was documented (Engel et al., 2015; Zheng et al., 2018). The aim of this study is to document the bacteriome of the *A. cerana* honey bees isolated from the gut of indigenous yellow and black races using culturable and non- culturable approaches (metagenomics sequencing).

MATERIALS AND METHODS

Healthy, live foraging worker honey bee samples of *Apis cerana* were collected from two locations of the Tamil Nadu state of India during the period of January, 2022 to March, 2022. One set of hill race bee samples were collected from the mountain ranges in Burliar (in Coonoor Ghat Road at an altitude of 830 metres) and the other set of bee samples were from the plains of Coimbatore (411m altitude) district of Tamil Nadu from the apiary situated at the Tamil Nadu Agricultural University. The whole alimentary canal of bee samples was aseptically dissected at the Molecular Ecology lab at the Centre for Plant Molecular Biology and Biotechnology, TNAU. The bee thorax was stabbed with a sterile needle tip and was pinned on wax. With the use of sterile micro forceps, the complete alimentary canal was gently pulled off in a single motion (Coleman et al., 2007). The dissected guts were transferred to PBS and immediately stored at −20°C for experimental studies (Anjum et al., 2018).

The bee gut samples (n = 5) were homogenised in 100 µl PBS using a micro pestle. Different dilutions (i.e., 1/10, 1/100, and 1/1,000) of this composite homogenate were made, and 10 µl aliquots each of the diluted samples were inoculated aerobically into three different media, namely, Nutrient agar (NA), De Man, Rogosa and Sharpe (MRS) agar and Luria Bertani (LB) agar and incubated for at 37°C for 18-24 hours. The bacterial colonies grown on the plates were enumerated and selected based on different colony morphologies. The distinctive colonies in master plates were repetitively sub-cultured to obtain pure bacterial colonies.

For the isolation of DNA, the CTAB method of extraction was utilised (Ellegard and Engel, 2019). The dissected guts of *A. cerana* bees were placed in 1.5 ml tubes containing 200 µl CTAB (lysis buffer) to homogenise the tissues using the micro pestle. The homogenised tissue was then placed in a water bath at 65°C for 45 min to 1 hour. The incubated samples are then centrifuged at 10,000 rpm for 10 min and the supernatant is placed in a new 1.5 ml tube. Next, chloroform: isoamyl alcohol (24:1) was added to the tube, thoroughly mixed by inversions and the next round of centrifugation was done at 12000 rpm for 12 min. The upper aqueous layer (supernatant) was removed and placed in a new 1.5 ml tube, with the addition of 300 µl isopropanol and 15 µl sodium acetate to it. The resultant mixture was stored overnight at -20°C. Next

day, the samples were centrifuged at 12000 rpm for 12 min and then the supernatant was removed carefully, leaving the pellet behind in the Eppendorf tube. The tubes were air dried until all traces of isopropanol are dried and the pellet was then dissolved in sterile water (50 µl) and stored at -20 °C for further use.

The 16S rRNA gene of all the isolates was amplified using the universal bacterial primers 8F and 1492R, which covered almost the full length of the gene. The PCR cycle consisted of an initial denaturation step at 94 °C for 2 min followed by 35 cycles of denaturation, annealing and extension at 94°C for 30 sec, 55°C for 30 sec and 72°C for 2 min, respectively. The final extension was kept for 10 min at 72°C. The PCR products were then separated on 1.5% agarose gel. The PCR bands were visualised under the UV-Trans illuminator and documented using documentation unit (UVITEC CAMBRIDGE). Amplicon size of 1.5 kb was obtained and the products were sent for Sanger sequencing to Biokart Pvt. Ltd., Chennai. The resultant sequences in the form of FASTA files for both the forward and reverse primers were used to perform homology search using the BLASTn programme at the National Centre for Biotechnology Information.

The ~1500 bp 16S rRNA gene comprises nine variable regions interspersed throughout the highly conserved 16S sequence. Using an *in-silico* dataset of sequences taken from public databases we show that commonly targeted 16S sub-regions, such as V3-V4, are unable to match the taxonomic accuracy achieved when sequencing the full 16S gene. Using long-read sequencing (V1-V9) of mock and in-vivo communities, it was demonstrated that it was possible to accurately resolve the divergent copies of the 16S gene that exist within the same genome (Johnson et al., 2019). In the present study, metagenomic sequencing (V1-V9) was done by Syngenome (OPC) Private Limited, Coimbatore. The DNA samples from the worker honey bees of both plain and hill races were used for metagenomic sequencing of 16S rRNA. Samples were subjected to both quantitative and qualitative analysis by nano drop method and agarose gel electrophoresis (1% agarose/ TAE), respectively. A total of 50 ng of good quality DNA was then used for nano pore library preparation for 16S rRNA gene amplification using V1-V9 specific primers and LongAmp (NEB) Taq 2X master mix. The expected amplicon length was ~1.5 kb which was confirmed by agarose (1%)/ EtBr gel electrophoresis. The PCR products were purified using 1.6x Ampure XP beads (Beckmann Coulter, USA).

A total of ~50 ng from each amplicon DNA was end-repaired (NEBnext ultra II end repair kit, New England Biolabs, MA, USA); cleaned up with 1x AmPure beads (Beckmann Coulter, USA). Barcoding adapter ligation (BCA) was performed with NEB blunt/TA ligase (New England Biolabs, MA, USA) and cleaned with 1x AmPure beads. Qubit quantified adapter ligated DNA samples were barcoded using PCR reaction (LongAmpTaq 2x New England Biolabs, MA, USA) and cleaned up with 1.6x AmPure beads (Beckmann-Coulter, USA). Qubit quantified barcode ligated DNA samples were pooled at equimolar concentration and end-repair was performed using NEBnext ultra II end repair kit (New England Biolabs, MA, USA). End-repaired DNA was cleaned up with 1x AmPure beads. Adapter ligation (AMX) was performed for 15 minutes using NEB blunt/ TA ligase (New England Biolabs, MA, USA). Library mix was cleaned up using Ampure beads and finally eluted in 15 µl of elution buffer. Sequencing was performed on MinION platform (Oxford Nano pore Technologies, Oxford, UK located at Syngenome Technologies, Chennai) using SpotON flow cell R9.4 (FLO-MIN106) in 8h sequencing protocol on MinKNOW (version 22.03.2, ONT) with live base calling enabled with default parameters. Nanopore raw reads ('fast5' format) were base-called ('fastq' format) and de-multiplexed using Guppy v6.0.6.

RESULTS AND DISCUSSION

Due to the importance of the bacteriome in the host health and development (Gilliam, 1997; Vojvodic et al., 2013; Moran, 2015; Zheng et al., 2018; Wu et al., 2021), this study analysed the gut bacteriome of plain and hill races of the Indian honey bees (foraging worker bees). One set of samples were the domesticated bees in the multiflora vegetation and the other set of samples were collected from the mountain ranges located near Coimbatore. Based on the colony morphology and characteristics, bacterial isolates underwent 16S rRNA gene amplification followed by sequencing. Consequently, the obtained sequences were analysed through BLAST for identification of the species (Wu et al., 2014; Anjum et al., 2018; Miroslava, 2019). In agreement with the previous studies (Disayathanoowat et al., 2012; Mathialagan et al., 2018; Anjum et al., 2018), the major phyla contributed was Proteobacteria followed by Firmicutes. The most probable genera isolated by a culturing approach were *Apibacter, Citrobacter, Lactobacillus* and *Enterobacter* in the honey bees. Apart from these other genera reported

were *Pseudomonas, Streptococcus, Lactococcus* and *Enterococcus* (Table 1). Prevalence of *Pseudomonas* has been observed in the Hill race honey bees gut microflora, which was found to have an important role in the spread of plant pathogens which causes plant diseases (Pattemore et al., 2014; Anjum et al., 2021). In addition, reports on the whole microbiome also dictate the same observation, where *Pseudomonas* SPS is being harbored by the carpenter bees (Subotic et al., 2019) and wild bees (Shell and Rehan, 2022).

The DNA was isolated from the guts of the 20 worker bees. The DNA isolates were subjected to 16S rRNA V1-V9 region sequencing. A total of 3,88,947 reads were generated from the nano pore sequencing after quality filtration with the average length of the reads for the plainer race was 1432bp and for the hill race was 1466 bp. The processed reads were analysed for taxonomic classification using the QIIME2 (version QIIME2-2021) and the number of OTUs revealed were 48 with 97% identity at species level.

The gut microbiota was relatively constant across the two geographical regions. Four major phyla of the bacteria, including Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes were detected in both plainer and hill race worker bee samples. However, Gemmatinodota and Cyanobacteria phylum were also reported to a small extent. Amongst these, proteobacteria accounted for about 60% in the hill race and up to 80% of relative abundance in the plain race (Fig. 1). Proteobacteria was found to be the most dominant bacterial phylum reported in this study for both the culturable as well as the non culturable approaches. Actinobacteria was the second abundant with around 30% presence in the hill race but was not reported abundant in the plain samples, followed by Firmicutes

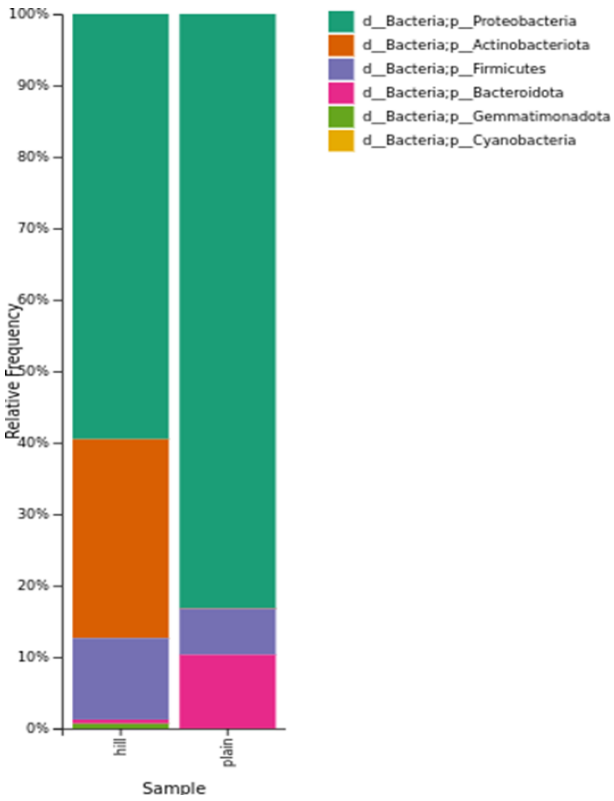


Fig. 1. The bacteriome classification at second level of taxonomy with Proteobacteria being the most dominant phyla reported in both hill and plain race followed by Actinobacteria, Firmicutes, Bacteroidota, Gemmatimonadota and Cyanobacteria phyla.

of around 10 to 15% and lastly Bacteroidetes with more active presence in the plain race as compared to hill race honey bees. Gemmatinodota and Cyanobacteria were reported very less. Though a change in proportion (was observed in the relative abundance of the various phyla between the present study and the previous research but the results were found in alignment with the former gut microbiome studies for honey bees (Disayathanoowat

Table 1. Characterisation of the culturable bacteria from the gut of the honey bees based on the Colony structure (elevation, texture, edge and colony colour) and 16S rRNA gene amplification.

Bee species	Caste and Pooled Compartment	Colony Morphology				16S rRNA gene
		Elevation	Colony texture	Edge	Colony Color	
A. cerana (Plain race)	Worker; Whole gut	Raised	Smooth, shinny	Round	Whitish	*Lactobacillus*
		Flat	Course	Rough	Creamy	*Enterococcus*
		Raised	Smooth	Round	Creamy	*Apibacter*
		Raised	Smooth	Round	Whitish	*Citrobacter*
A. cerana (Hill race)	Worker; Whole gut	Raised	Smooth	Round	Whitish	*Pseudomonas*
		Raised	Smooth	Round	Whitish	*Apibacter*
		Raised	Shiny	Round	Whitish	*Lactobacillus*

Table 2. Classification of the *A.cerana* gut bacteriome (using the metagenomic approach targeting the V1-V9 region of the 16S rRNA gene.)

Phylum	Class	Order	Family	Genus	Species
Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	*Noviherbaspirillum*	*Gamma proteobacterium*
Actinobacteria	Alphaproteobacteria	Orbales	Neisseriaceae	*Massillia*	*Snodgrasella alvi*
Firmicutes	Actinobacteria	Pseudomonadales	Comamonadaceae	*Snodgrassela*	*Terrabacter tumescens*
Bacteroidota	Bacilli	Sphingomonadales	Burkholderiaceae	*Ramlibacter*	*Enterobacteriaceae*
Gemmatimonadota	Bacteroidota	Acetobacterales	Orbaceae	*Caenimonas*	*Spinghomonas spp.*
	Gemmatimonadetes	Caulobacterals	Moraxellaceae	*Variovorax*	*Phyciococcus ochagensis*
		Rhizobiales	Sphingomonadaceae	*Polynucleobacter*	*Ceanimones*
		Micrococcus	Acetobacteraceae	*Gilliamella*	*Massillia spp.*
		Lactobacillus	Caulobacteraceae	*Frischella*	*Bifidobacterium asteroids*
		Bacflavobacteriales	Beijerinckiaceaea	*Orbaceae*	*Arthrobacter spp.*
		Bacteroidetes	Intrasporangiaceae	*Acinetobacter*	*Acinetobacter spp.*
		Micrococceals	Micrococcaceae	*Sphingomonas*	*Enterobacter cloacae*
		Frentials	Bifidobacteriaceae	*Altererythrobacter*	*Apibacter mensalis*
		Proionibacterials	Microbacteriaceae	*Belnapia*	*Bifidobacterium indicum*
		Bifidobacterials	Lactobacillaceae	*Neokomagataea*	*Variovoraxparidoxus*
			Weeksellaceae	*Phenylobacterium*	*Klebsiella pneumoniae*
			Dysogononomedaceae	*Blastococcus*	*Gilliamella apicola*
			Gemmatinonadaceae	*Pseudoarthrobacter*	*Pseudoarthrobacter sulfonivorans*
				Physicoccus	*Lactobacillus hesingborgensis*
				Terrabacter	*Pedococcus dokdenensis*
				Arthrobacter	*Enterobacter rogenkampii*
				Lactobacillus	*Uncultured bacterium*
				Apibacter	
				Elizabethkingia	
				Dysgonomonas	
				Roseisolibacter	
				Gemmatimonas	

et al., 2012; Dong et al., 2020; Duong et al., 2020; Tola et al., 2020; Lombogia et al., 2020).

Amongst these, the most dominant family of bacterial diversification was reported to be the Oxalobacteraceae family in the hill race with around 30% of abundance and the plain samples were more dominated by the Orbaceae family with 80% abundance (Table 2; Fig. 2). The hill race samples showed more variation at family level as compared to plain race samples. The hill race also revealed Lactobacillaceae, Sphingomonadaceae, Intrasporagiaceae, Neisseriaceae families with good relative abundance. The plain race samples showed less abundance of families in the bacteriome with most dominance by Orbaceae and few relative abundances of Lactobacillaceae, Neisseriaceae and Weeksellaceae families. Eneterobaceae and Bifidobacteriaceae families were also in the list with more presence in the hill race honey bees. The presence of low Biforbacteriaceae and Lactobacillaceae was speculated to point towards the presence of pathogens in the gut (Lombogia et al., 2020).

The phylum Proteobacteria dominated both at the genus and the species level (Fig. 3). At the genus level within Proteobacteria, for the hill race of honey bees, a good number of genera are reported with no clear dominant members. The present study reported the *Noviherbaspirillum, Sphingomonas, Snodgressela,* and *Masillia*, *Gilliamella* dominated the most with about 80% abundance in the plain race. The phylum Firmicutes was dominated by the *Lactobacillus* genus, with these genera being the second highest at the genus level of diversification. The presence of lactic acid bacteria as *Lactobacillus* species has been reported to produce compounds that are antimicrobial in nature.

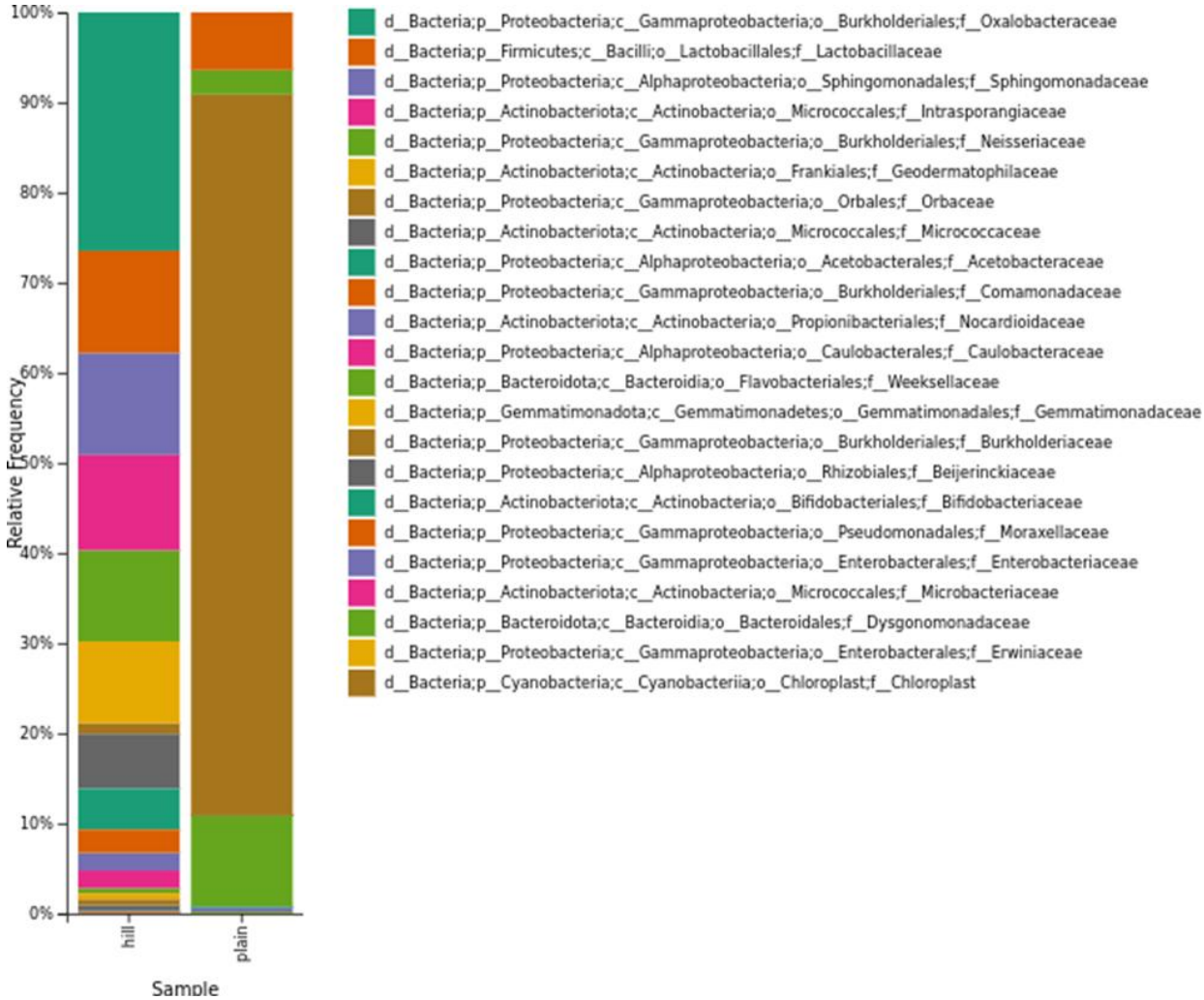


Fig. 2. The bacterial composition in the gut of *Apis cerena* at the genus level with highest abundance of *Oxalobacteraceae* and *Orbaceae* in the hill and plain race honey bees respectively

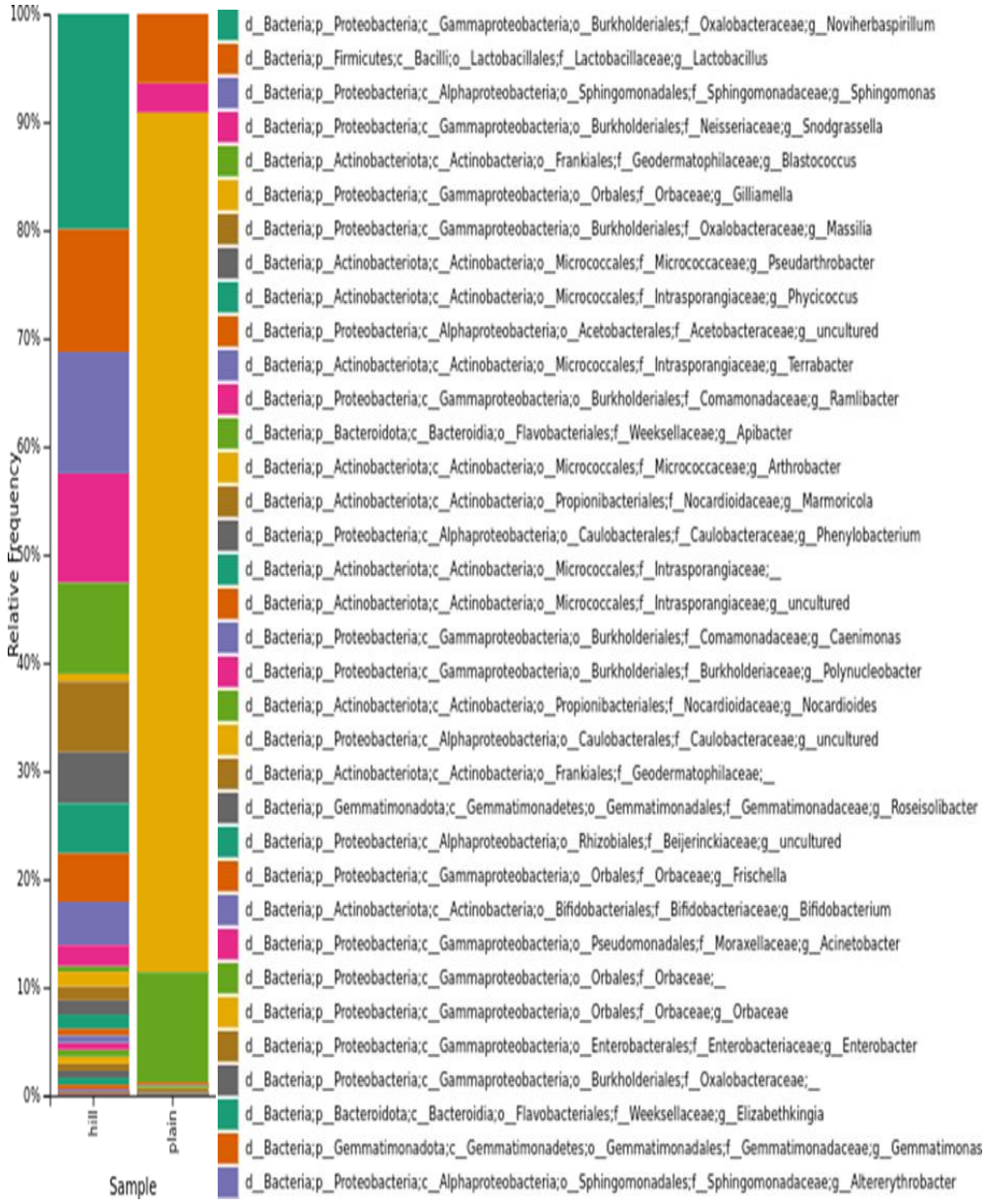
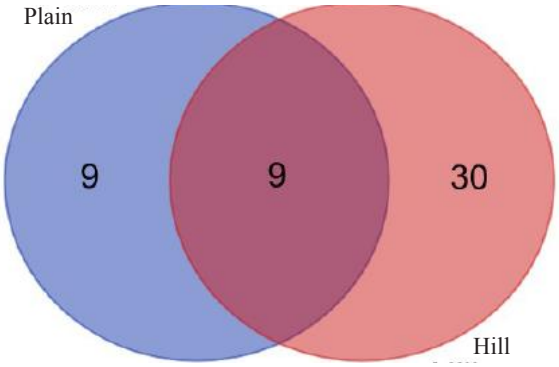


Fig. 3. The composition of the gut bacteriome. The microbiome was dominated by *Noviherbaspirillum* in the hill race and by *Gilliamella* in the plain race honeybees.

The indication of the presence of *Lactobacillus* in the present study is an indication of the usage of these as probiotics in the gut of the honey bees (Lombogia et al., 2020). The abundance of such probiotics can be used as an important aspect to bee health (Lombogia et al., 2020). For the Actinobacteria phylum, the most occurring genera were constituted by *Blastococcus* for the hill race. Apart from *Gilliamella*, the plain race bees also reported *Lactobacillus, Snodgressela* and *Blastococcus* to some extent. The number of OTUs (Operational Taxonomical Units) determined in our study at the genus level for the bacterial composition has been displayed in Fig. 4. A total of 48 OTUs were determined, nine unified OTUs belonging to the plain race bees and 30 OTUs were from the hill race honey bees. Common OTUs for both the races were only nine in number which were part of both the races. More distinctive level of variation was observed in the hill race honey bees as compared to the plain race. The alpha diversity in terms of the OTUs was determined using the Shannon vector algorithms. Generally, the Shannon index ranges from 1.0 to 3.5 (Lombogia et al., 2020) and for our study the Shannon entropy for Hill samples was 2.8 and 1.2 for Plain race honey bees



Hill race	OTUs	Plain race	OTUs
Noviherbaspirillum	5119	*Enterobacter*	53
Sphingomonas	2933	*Salmonella*	2
Phycicoccus	1206	*Neokomagataea*	1
Massilia	1641	*Klebsiella*	11
Polynucleobacter	177	*Escherichia-Shigella*	1
Caenimonas	159	*Dysgonomonas*	25
Arthrobacter	359	*Variovorax*	1
Terrabacter	1059	*Pantoea*	1

Fig. 4. Unified OTUs reported in the hill and plain race honey bees at the genus level from Coimbatore, Tamil Nadu

(Fig. 5). These results showed good diversity in the Hill race bees but a low microbiome diversity in the plain race honey bees. As the value of OTUs increases, the distribution of the individuals in the taxa becomes more even (Lombogia et al., 2020).

The core members of the bee gut microbiome observed have been highlighted herein and these are conserved across the different geographies. Proteobacteria was the most dominated phylum reported in the present work, along with Firmicutes, Actinobacteria and Bacteroidetes. The community structure in the gut indicates the possible insight of how these bacteria affect the host health. This study concludes that the Hill race has a diverse nectar and pollen source from a wide array of bee pasturage which is absolutely lacking in the plain race of honey bee within the city campus of Coimbatore. So, the hill and plain race are not distinct but the food source makes the difference. Present results highlight the importance of future studies on honey bees in India to harness the potential of this microbiome for improving bee health. More studies will contribute to a better understanding of the role of single members to the health of the host. A better understanding of the indigenous bee can also help with combating the global issues such as the decline in the bee population and the enhancement of pollination services. Honey bees can be used as good tractable model systems that can offer numerous parallels to the human gut microbiota.

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AUTHOR CONTRIBUTION STATEMENT

S. Mohankumar, N Saranya conceived and

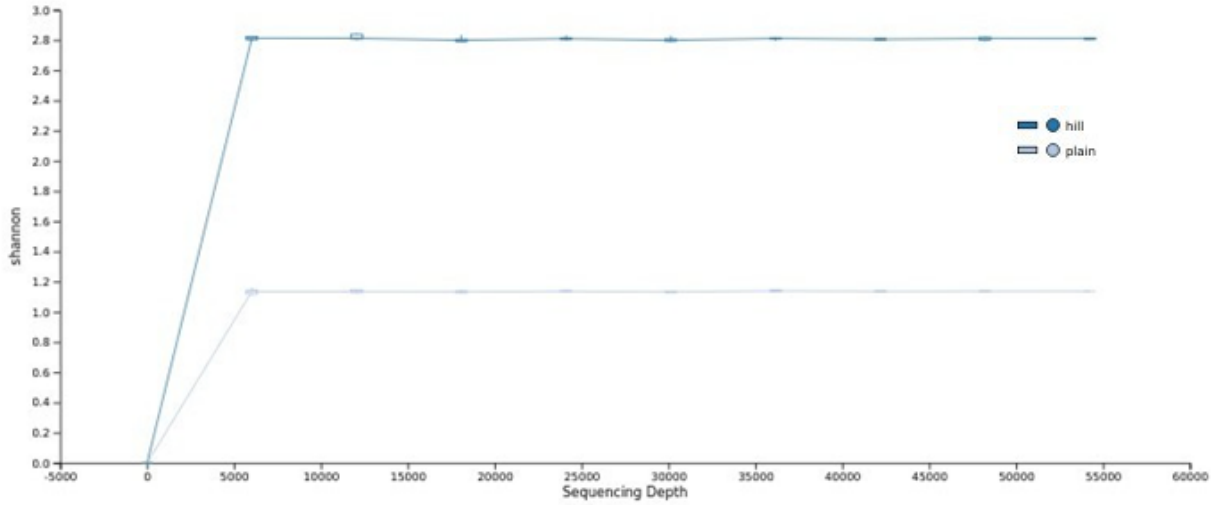

Fig. 5. Shannon vector Algorithm for hill and plain race at 2.8 and 1.1 index respectively displaying good bacterial diversity for hill race honey bees as compared to plain race bees

designed the research. Akanksha Thakur conducted the experiments and wrote the manuscript. S. Nakkeeran, M R Srinivasan and S. Subramaniam contributed to the samples and analysed the results. All authors read and approved the manuscript.

CONFLICT OF INTEREST

Authors have declared no competing interests.

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SURPLUS FEEDING REVERSES WORKER OVARIAN SUPPRESSION AND DISRUPTS SOCIAL COHESION IN QUEEN-RIGHT WASP COLONIES

Shantanu P Shukla* and Raghavendra Gadagkar[1]

Molecular Reproduction, Development and Genetics;
[1]Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, Karnataka, India
*Email: sps@iisc.ac.in (corresponding author): ORCID ID 0000-0002-4093-2772

ABSTRACT

Nutrition is an important component of oogenesis and ovarian development in insects. In social insect colonies where a large proportion of females are sterile, suppression of reproduction caused by differential acquisition and retention of nutrition has been hypothesized to cause worker sterility. This study, using the primitively eusocial wasp *Ropalidia marginata* (Lepeletier) (Hymenoptera: Vespidae), show that worker ovarian development in naturally foraging colonies is more similar to experimentally food-limited colonies than to surplus-fed colonies, indicating nutritional castration in workers whose ovaries are inhibited by food availability. Further, experimental provisioning of surplus nutrition led to higher ovarian development in workers as compared to naturally foraging and food-limited workers. Surplus feeding also led to higher nest desertion by workers, leading to a breakdown of the colony's social structure, whereas food-limited colonies retained workers.

Key words: Nutrition, eusociality, oogenesis, worker sterility, caste differentiation, *Ropalidia marginata*, reproduction, suppression, ovarian development, nutritional castration, food availability, surplus feeding

Social insect colonies are characterized by reproductive division of labour. Reproduction is monopolised by one (or very few) females of the queen caste, and a large majority of females are sterile in the form of the worker caste such that queens' ovaries are well-developed and worker ovaries are arrested or atrophied at early stages of differentiation. Genetic as well as environmental factors are considered important in regulating ovarian development in social insect colonies. One important environmental factor is nutrition. The trophic castration hypothesis (Marchal, 1897) and the alimentary castration hypothesis (Roubaud, 1916) proposed that differential larval nutrition led to 'undernourished larvae' that developed into adults with 'underdeveloped ovaries'. The 'nutricial' castration hypothesis (Marchal, 1897) attributed worker sterility to energetically demanding worker roles, especially brood care by nursing workers (Hunt, 1991). Similarly, nutrient depletion due to foraging, and other worker behaviour may influence worker sterility and outcomes of caste differentiation (Chandrashekara and Gadagkar, 1991; Krishnan et al., 2021). Natural inter-individual variation in nutrition created by unequal availability and distribution of food amongst nest-mates (Hunt, 1994) and differential disposition to consume food in adult stages (Gadagkar et al., 1991; Shukla et al., 2013) may further compound differential reproductive development in queens and workers leading to the evolution and maintenance of sociality. Variation in levels of larval nutrition and maternal manipulation influence lipid reserves, ovarian development and reproductive caste differentiation in many wasp societies (Berens et al., 2015; Gadagkar et al., 1991; Gadagkar et al., 1990; Hunt and Dove 2002; Jandt et al., 2017; Judd et al., 2015; Suryanarayanan et al., 2011). Adult nutrition on the other hand can affect brood survival, nest growth, body size and fat deposition (Karsai and Hunt 2002; Mead et al., 1994; Toth et al., 2009). Social insect colonies have inherent mechanisms that can regulate the nutritional status of its members (viz., through dominance-subordinate behaviours, or through division of labour involving energetically costly behaviours such as foraging), and it is not entirely clear what levels of variation in ovarian development prevail in natural colonies and if natural colonies are more similar to starved or well-fed colonies in this respect? Also, if nutritional castration does indeed limit worker reproduction in social insect colonies, can surplus nutrition rescue worker ovarian development if excess food is made available?

This study uses the primitively eusocial wasp *Ropalidia marginata* to answer both the above questions. Importance of larval nutrition in worker sterility, egg laying and nest-founding has already been established in *R. marginata* (Gadagkar et al., 1990;1991). Therefore,

this study describes 'nutritional castration' as an effect of adult nutrition on ovarian development, over and above the contribution of larval nutrition that is carried forward in wasps, without strictly adhering to the definition of 'castration' as provided by Marchal (1897) and Roubad (1916). Firstly, this study describes the ovarian development in natural free-foraging colonies and compares it with colonies that were limited by food and those that were fed surplus food. Secondly, this tests if surplus nutrition can rescue worker ovaries, by leading to higher ovarian development in queen-right workers. Additionally, this study predicts that surplus nutrition should lead to higher worker dispersal away from their natal colonies, either to explore opportunities of direct reproduction, or because surplus nutrition precludes conditions necessary for forming social groups. In contrast, starvation should suppress ovarian development in workers, reinforce worker status and curtail female dispersal.

Ropalidia marginata (Lepeletier) is a primitively eusocial, tropical, perennial nesting wasp species where queens are the sole egg layers of a colony. Queens and workers are morphologically identical. Reproductive castes are temporary, and though workers have poorly developed ovaries in the queen's presence, they often replace queens to then develop ovaries and begin egg laying (Gadagkar et al., 1993). Wasps are capable of solitary nest initiation, and new nests can be founded singly or in groups (Shakarad and Gadagkar, 1995). Higher larval and adult nutrition significantly improve female ovarian development and egg laying when reared in isolated conditions, although with high variation (Gadagkar et al., 1991; Shukla et al., 2013). Queens are docile, and do not use aggression to regulate worker ovarian growth or colony labour, and worker ovaries may be inhibited through the queen's pheromone (Bhadra et al., 2010), or through energetically demanding activities such as foraging (Chandrashekara and Gadagkar, 1991). Starvation increases dominance behaviours in workers, which use it to signal hunger to nestmates and foragers, which then leads to higher rates of foraging (Bruyndonckx et al., 2006; Lamba et al., 2008).

MATERIALS AND METHODS

Experimental setup: Ovarian development was studied in natural colonies and laboratory-reared experimental colonies. Natural colonies: Seven *R. marginata* colonies located at the Indian Institute of Science, Bangalore, India (13°00' N, 77°32' E) were observed in a natural setting that allowed natural foraging activity. No nutritional supplementation of any form was provided experimentally. Experimental colonies: Eight *R. marginata* colonies were collected in Bangalore, India, brought to the laboratory and fixed in closed wooden cages (Gadagkar, 2001). Each cage housed a single wasp colony. Cages were closed to prevent wasps from foraging and bringing additional food to the colony but allowed for ventilation and ambient light to enter. The eight experimental close-caged colonies were further divided into two categories and randomly assigned to food-limited and food-surplus categories (four colonies each). From day one, when the cages housing the experimental colonies were closed, females were fed as per the following regimens for a total of ten days. Food-limited colonies: Maximum of two *Corcyra cephalonica* (Lepidoptera: Pyralidae) larvae was placed daily in a food tray inside each closed cage. None of the wasps were hand fed. Water, honey and soft wood were placed in all cages at all times. The number of *Corcyra* larvae consumed from the food tray was recorded. Food-surplus colonies: Each wasp present in the cage was individually hand-fed with as many *Corcyra* larvae as it accepted. This was performed thrice daily. Apart from hand feeding, ad libitum *Corcyra* larvae, honey, water and soft wood were kept in a food tray inside each closed cage at all times.

Behavioural observations: Wasps from natural and experimental colonies were marked with quick drying, non-toxic paints to assign unique colour codes for individual identification. Natural nests were observed for 20 hr in the natural setting and laboratory nests were observed for 10 hr (spread over four days and two days, respectively) as described before (Gadagkar, 2001). Behavioural observations were performed only after the wasps had finished eating the experimentally provided *Corcyra* larvae. All wasps from the experimental colonies were collected at the end of day 10. Food-surplus colonies consumed an average of 10.25 (s.d.± 4.63) *Corcyra* larvae/ day. Food-limited colonies consumed an average of 1.02 (s.d.± 0.25) *Corcyra* larvae/ day. Since foragers (or wasps in general) return to their colony after dusk, a night census was used to determine the proportion of nights that a wasp did not return to her colony, calculated as the number of nights spent outside the nest by the female (on the walls of the closed cage) divided by the total number of nights in the census.

Quantification of ovarian development: At the end of the experiment, all females were frozen at -20°C

until ovarian dissections. Number of mature oocytes, mean length of proximal oocytes, mean width of proximal oocytes, number of vitellogenic oocytes, and total number of oocytes was recorded for seven queens and 81 workers for natural colonies, and eight queens and 85 workers for the experimental colonies. Ovaries were measured in blind, i.e., the experimental treatment to which the wasp belonged to, was concealed during dissections. Ovarian measurements were subjected to principal component analysis (statistiXL, version 1.8) and principal component 1 scores (which explained 87.3% of the total variance) were used as an ovarian index for each female (Chandrashekara and Gadagkar, 1991).

Statistical analysis: To test for differences between ovarian development between feeding regimens, ovarian indices were used as the response variable, and feeding category (natural, food-limited, and food-surplus) and female caste (queen and worker) were used as categorical factors in a generalized linear model (GLM), analysed in R (R Core Team 2015) using the *multcomp* package (Bretz et al*.,* 2008). Post-hoc Tukey's pairwise comparisons between feeding treatments were performed separately for workers and queens.

RESULTS AND DISCUSSION

There was a significant effect of feeding regimen on ovarian development in females of naturally foraging, food-limited, and food-surplus colonies (GLM, F=4.27, p=0.015; Table 1). Ovarian indices of workers belonging to naturally foraging and food-limited colonies were similar to each other (GLM post-hoc test, p=0.825, Fig. 1; Table 2). Most workers from food-limited colonies as well as naturally foraging colonies had rudimentary ovaries, with no differentiating oocytes observed in them, arrested in early stages of ovarian development, and thus having low ovarian indices (Fig. 1). Workers from food-surplus colonies, however had significantly higher ovarian indices than naturally foraging workers and food-limited workers (GLM post-hoc tests, p= 0.009 and p= 0.049 respectively, Fig. 1) and thus suppression of worker ovaries was less effective in food-surplus colonies. The distribution of ovarian indices between food-surplus colonies and food-limited colonies differed significantly between workers (Fig. 2, Kolmogorov-Smirnov test, D= 0.30, p= 0.039) but not for queens (Fig. 2, Kolmogorov-Smirnov test, D= 0.75, p= 0.228). Workers from food-surplus colonies showed significant advances in ovarian development including higher number of differentiated oocytes, and higher number of vitellogenic oocytes that were not observed in workers from food-limited or natural colonies as reflected in their ovarian indices (Fig. 1, 2). There were no differences between ovarian indices of queens belonging to natural, food-limited, and food-surplus colonies (GLM post-hoc test, p=0.228, Fig. 1, Table 1).

The similarity between ovarian indices of naturally foraging and food-limited workers in this study indicated that workers in natural colonies might already be in the lowest physiological level of ovarian development as starvation did not further reduce ovarian development in food-limited workers. Natural, free-foraging *R. marginata* worker ovaries were therefore limited by food availability. Surplus nutrition in adults reversed the effects of nutritional

Table 1. Analysis of deviance table for generalized linear model (GLM) fit for the relationship between ovarian development (as estimated by the Ovarian Index), feeding regimens (naturally foraging, food-limited, and food-surplus colonies) and caste of the female (queen or worker)

	Df	Deviance	Residual degree of freedom	Residual deviance	F-statistic	p-value
NULL			180	710.23		
Feeding regimen	2	7.65	178	702.58	4.27	0.0153
Caste	1	544.37	177	158.20	609.05	<2e-16

Table 2. Results of post-hoc test of multiple comparison of means between each pair of feeding regimen, using Tukey's contrasts performed for GLM analysis for worker ovarian indices. Non-adjusted p-values reported

Pair of comparisons	Estimate	Standard error	z value	p-value
Natural - food surplus	-0.43	0.16	-2.59	0.009
Food limited - food surplus	-0.39	0.19	-1.96	0.049
Food limited - natural	0.03	0.18	0.22	0.825

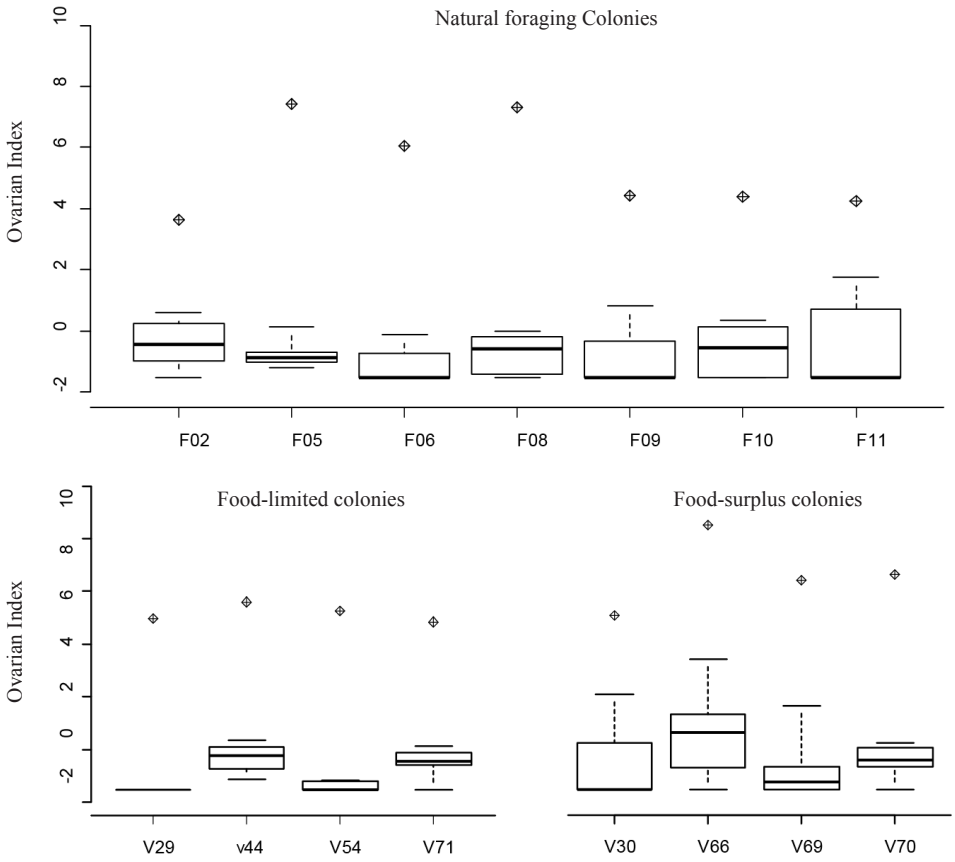

Fig. 1. Comparison of worker ovarian indices: workers from naturally foraging colonies and food-limited colonies did not differ in their ovarian indices. Workers from food-surplus colonies had higher ovarian indices than workers in food-limited and naturally foraging colonies. Queen ovaries did not differ between the natural, food-limited and food-surplus colonies (separate generalized linear models for queens and workers, post-hoc test, alpha= 0.05). Worker ovarian indices are plotted as boxplots indicating median values inside the boxes. Queen ovarian index for each colony is plotted above the boxplot as a diamond plus. X-axis indicates individual colony IDs

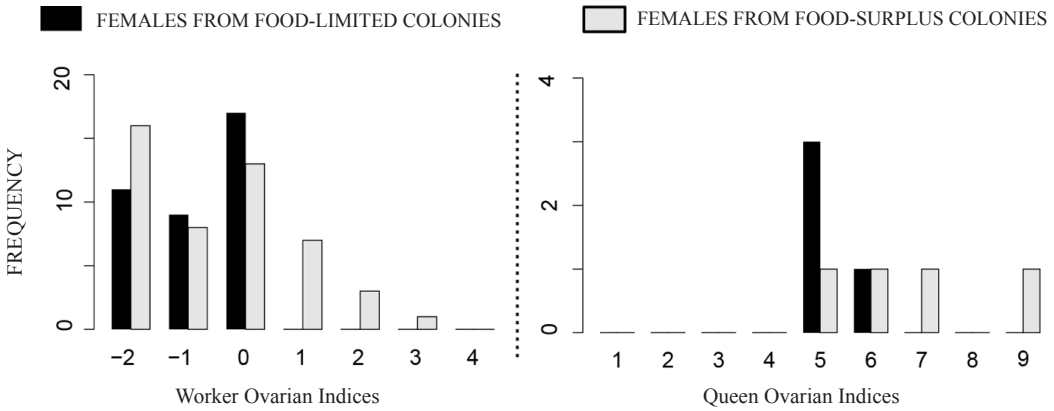

Fig. 2. Distribution of worker ovarian index (OI), a measure of the extent of ovarian development in individual females calculated using multiple ovariole parameters. OI values for food-limited colonies (black bars) were significantly different as compared to worker OI values for food-surplus (grey bars) colonies (Kolomogorov-Smirnov test, $p < 0.05$). Many surplus-fed workers belonged to higher OI classes that were absent in starved workers

castration, leading to higher ovarian development in workers under queen-right conditions. Since food-surplus and food-limited colonies differed only in their availability of food, whereas energetic demands in tasks pertaining to brood care and colony maintenance were minimal and comparatively similar between the two treatments, surplus adult nutrition was the most likely cause leading to the observed differences in ovarian development. Since queen ovarian development was not different between food-surplus and food-limited colonies, *R. marginata* queens may already be at the highest physiological limit of oogenesis, selected for maximising their reproductive output as the sole egg layer of their colony (Table 3). Further, queens could have high-energy reserves that sustain oogenesis even during times of nutrient deprivation. It is possible that further starvation could reduce ovarian growth in queens, but *R. marginata* queens are docile, and generally do not participate in dominance behaviours, or energetically expensive behaviours such as foraging, and this could be another mechanism by which the queens conserve nutrient expenditure so as to maximise investment in oogenesis.

It was found that food-surplus colonies experienced higher nest desertion as compared to food-limited colonies. The proportion of nights that workers did not return to their natal nest was significantly higher in food-surplus colonies than in food-limited colonies (Fig. 3, Wilcoxon rank sum test, W= 608.5, p= 0.002). Thus, surplus-feeding increased nest desertion indicated by the number of wasps that did not return to their natal nests. There is evidence that excess fed *R. marginata* workers also show reduction in colony associated behaviours such as larval feeding, foraging, and dominance to nestmates (Bruyndonckx et al., 2006; Lamba et al., 2008). Such reduced worker activity may be a preliminary effect of surplus nutrition, and prolonged excess feeding (such as in this study) could eventually lead to nest desertion, and disintegration of the overall colony structure. Starvation on the other hand ensured that more females remained back and continued on their nests as workers, consistent with

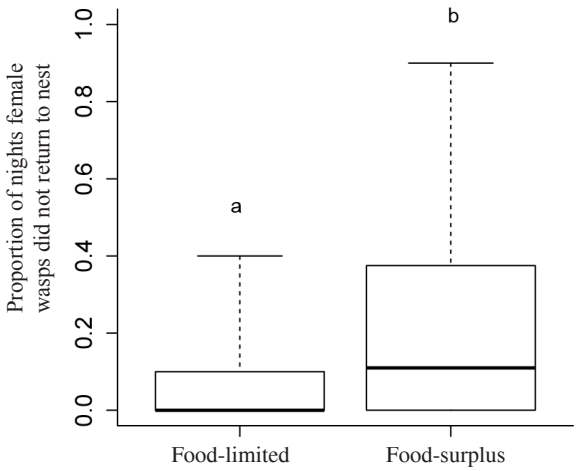

Fig. 3. Excess fed workers spent a significantly greater proportion of nights away from their natal nest as compared to starved workers (Wilcoxon rank sum test, $p<0.05$). Workers that did not return to their nest were observed far from the nest, on the walls of the closed cage in which the nest was housed

earlier studies where starved *R. marginata* colonies show increased nest maintenance and care under starvation (Lamba et al., 2008). Surplus nutrition thus disintegrated colony structure, whereas starvation was associated with group cohesion. Surplus-feeding was accompanied by natural queen turnover in one colony, where one of the workers behaviourally dominated and drove away the existing queen, whereas separate nest founding by female(s) was observed in another surplus-fed colony. Taken together, these results suggest that surplus nutrition facilitates workers to overcome ovarian suppression and to desert natal nests. *Ropalidia marginata* wasps can desert colonies in groups, where multiple foundress colonies can be founded by two to 22 individuals (Shakarad and Gadagkar, 1995), which could explain why a large number of females in excess-fed colonies deserted their nests. Similar effects on colony demographics also occur in *Polistes* wasps, where honey-supplemented colonies produced higher proportion of reproductives and led to a lesser number of workers remaining on the nest (Hunt and Dove, 2002).

Differential nutrition in social insects, especially in

Table 3. Results of post-hoc test of multiple comparison of means between each pair of feeding regimens, using Tukey's contrasts performed for GLM analysis for queen ovarian indices. Non-adjusted p-values reported

Pair of comparisons	Estimate	Standard error	z value	p-value
Natural - food surplus	-1.30	0.82	-1.57	0.115
Food-limited - food surplus	-1.49	0.93	-1.59	0.110
Food limited - natural	-0.18	0.82	-0.22	0.819

the larval stages can regulate gene expression and alter developmental trajectories committing or predisposing females to certain reproductive roles as adults (Berens et al., 2015; Gadagkar et al., 1991; Gadagkar et al., 1988; Jandt and Toth 2015; Judd et al., 2015; Karsai and Hunt, 2002; Kucharski et al., 2008). However, the quality and quantity of larval nutrition by itself is inadequate to explain the complex course of caste differentiation in reproductively totipotent primitively social wasps (Agrahari and Gadagkar, 2003; Gadagkar, 1991; 2001; Mead et al., 1994; Shukla et al., 2013; Shukla et al., 2014; Solis and Strassmann 1990; Suryanarayanan et al., 2011; Wheeler 1986). Asymmetries in adult nutrition arising through the disproportionate acquisition or retention of food, *viz*. through snatching of food by certain individuals from incoming foragers (Gadagkar, 2001), nutrient transfer through aggressive adult-adult trophallactic interactions (Pardi,1948), self-feeding propensities either in isolation (Shukla et al., 2013; Tibbetts et al., 2011) or in the context of natal nests (Brahma et al., 2018; Judd et al., 2015; Markiewicz and O'Donnell, 2001) and the nutritional costs of worker behaviour (Hunt, 1991; Marchal, 1897; Markiewicz and O'Donnell, 2001; Richards, 2021) can influence worker ovarian development and probabilities of future nest founding. This current study therefore aimed at reversing potential nutritional deficiencies (and the arising nutritional castration) in queen-right workers and comparing ovarian development in workers of food-surplus colonies with food-limited and naturally foraging colonies. It was found that post-imaginal nutritional supplementation reversed ovarian suppression in queen-right workers and resulted in the loss of colony cohesion. Based on these results, it can be suggested that access to surplus nutrition and the resulting increase in ovarian development and nest desertion promotes colony fission and queen-turnover. Surplus-fed deserter workers should eventually explore reproductive options either in groups (as multiple foundresses) or individually (as single foundresses) at the expense of maintaining colony social cohesion (Krishnan et al., 2021). Patterns of worker- foundress transitions and of queen turnovers should be clearer when deserter wasps experience more optimal spatial conditions, as demonstrated in a followup study (Krishnan et al., 2021). Seen in the broader context of the effects of nutrition on body size, ovarian development, egg laying, and gyne production in social wasps (Gadagkar et al., 1991; Hunt and Dove, 2002; Karsai and Hunt, 2002; Shukla et al., 2013), this study further highlights the importance of nutrition in regulating female ovarian development, as well as group cohesion in social insects. Nutritional control of female reproductive regulation may thus, not only affect mechanisms of caste differentiation in social insects but may also be a binding force for the maintenance of sociality.

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AUTHOR CONTRIBUTION

SPS and RG designed study and co-wrote the paper and SPS conducted the study. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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DISTRIBUTION AND MITOTYPE DIVERSITY OF *BEMISIA TABACI*

Ramesh K B, Mahendra C[1], Kelageri S S, Rajna S and Subramanian S*

Division of Entomology, [1]Division of Molecular Biology and Biotechnology,
ICAR-Indian Agricultural Research Institute (IARI), New Delhi 110012, India
*Email: subramanian@iari.res.in (corresponding author): ORCID ID: 0000-0001-8337-9666

ABSTRACT

Comparative assessment of the mitochondrial cytochrome oxidase I (mtCOI) gene sequence uncovers the *Bemisia tabaci* mitotypes. The current study aims to define *B. tabaci* mitotypes found in various agro ecological zones across India. Phylogenetic analysis of 13 samples from various agroclimatic regions revealed presence of four mitotypes: Asia 1, Asia II 1, Asia II 5 and Asia II 7. Among, Asia II 1 was found to be abundant and widespread distribution in the thirteen locations studied. From the 13 sequences examined, 7 haplotypes viz., three of Asia II 1, two of Asia II 5, each one of Asia 1 and Asia II 7 were identified. Nucleotide diversity was observed to be greater in Asia II 5 (0.00388) than in Asia II 1 (0.00258), and haplotype diversity was observed to be greater in Asia II 5 (1.000) than in Asia II 1 (0.600).

Key words: *Bemisia tabaci,* mitotypes, Asia 1, Asia II 1, Asia II 5, Asia II 7, haplotypes, mtCOI, nucleotide diversity, Clustal W, distribution

Bemisia tabaci (Gennadius), (Hemiptera: Aleyrodidae) is a cosmopolitan and polyphagous insect pest and among the most devastating insect pests of the twentieth century. *B. tabaci* is known to attack over 700 plant species and to vector around 100 plant viruses causing huge economic loss in many crop plants. It is designated as a complex of morphologically similar but physiologically, biochemically and genetically dissimilar species (Rehman et al., 2021; Khamis et al., 2021). Currently, there are 45 cryptic species of *B. tabaci* reported globally and 9 have been identified in India based on 4% genetic divergence in the mitochondrial cytochrome oxidase subunit I (mtCOI) sequences (Rehman et al., 2021). Bayesian inference analysis recognizes 45 well established cryptic species which were grouped into 11 major clades viz., Asia, Australia, China, Italy, Japan 1, Japan 2, Middle East Asia, New world, Sub-Saharan, Sub-Saharan Africa 7, Sub-Saharan Africa 10 and Uganda. Commonly these clades are recognized as genetic groups (Jiu et al., 2017; Mugerwa et al., 2018; Rehman et al., 2021). Similarly, the Bayesian inference analysis reveals 9 established cryptic species of *B. tabaci* in India namely Asia I, Asia II 1, Asia II 5, Asia II 7, Asia II 8, Asia II 11, Asia IV, MEAM1, and China 3. The cryptic species viz., Asia 1, Asia II 5, and Asia II 8 are predominant in southern part of India whereas Asia II 1 and Asia I was found prevalent in northern and central parts of India. Western India has the highest number of *B. tabaci* Asia I and Asia II 7 records. In Eastern India, *B. tabaci* Asia I, Asia II 1, and Asia II 5 were most common. The most recent sequence data showed a rise in *B. tabaci* China 3 occurrence in eastern India with its wider host range (Rehman et al., 2021; Chowda-Reddy et al., 2012; Ellango et al., 2015; Prasanna et al., 2015; Rekha et al., 2005; Singh et al., 2012).

The genetic divergence among cryptic species and *B. tabaci* genetic groups was 4.1–23.4% and 11.7–21.2%, respectively (Rehman et al., 2021). The intraspecific diversity *B. tabaci* has been characterized as biotypes before being identified as a cryptic species based on mitochondrial cytochrome oxidase subunit I (mtCOI) (De Barro et al., 2011). The genetic difference between these cryptic species was determined to be 3.5% but with far more sequence divergence between individuals of the same species, the cryptic species categorization cut-off value was reset to 4% (Dinsdale et al., 2010; Lee et al., 2013). Asia II 1 genotype was found to be more prevalent, with widespread distribution over tropical, subtropical, and temperate zones of Indian subcontinent and showed the highest haplotype diversity, whereas Asia 1 genotype indicated the highest nucleotide diversity (Prasanna et al., 2015). The most common genetic groups in Delhi are Asia II 1 and Asia II 7 (Hashmi et al., 2018; Ahmed et al., 2010; Prabhulinga et al., 2020; Hameed et al., 2012). A deeper understanding of the distribution patterns of genetic variation, species diversity, haplotype variability is very much essential to design or formulate effective management approaches for the continually evolving *B. tabaci* cryptic species

complex. The primary goal of this research was to document the identity, spread, and diversity of *B. tabaci* species on Cotton from various agro-climatic regions in India using the mitochondrial subunit.

MATERIALS AND METHODS

Whiteflies were collected from 13 agroclimatic regions viz., Banswara: Rajasthan, Sri Ganganagar: Rajasthan, Amravati: Maharashtra, Hisar: Haryana, Indore: Madhya Pradesh, Bathinda: Punjab, Meerut: Uttar Pradesh, Guntur: Andhra Pradesh, Raipur: Chhattisgarh Jalandhar: Punjab, Coimbatore: Tamil Nadu, Pusa: New Delhi. A hand-held aspirator was used to capture adult whiteflies from cotton plants. Thereafter, the collected whiteflies shifted to 1.5 ml eppendorf tubes containing 70% ethanol. The collected samples were preserved in -20°C until further analysis. A single whitefly was used to isolate DNA. Each whitefly was thoroughly cleaned in autoclaved water. The washed whiteflies were then homogenized with a hand-held homogenizer (Sigma Aldrich) and DNA was extracted using the DNASure Tissue mini kit (Qiagen#NP-61305) according to the manufacturer's instructions. The extracted DNA treated with RNase (0.1g/ L) for 45 min at 37°C. The DNA was then be examined on a 0.8 % Agarose gel containing ethidium bromide (0.5g/ ml). The quantified DNA was used for further PCR analysis. The universal primers C1-J-2195 (5'-TTGATTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5' - TCCAATGCACTAATCTG CCATATTA-3') were used to amplify a partial mtCOI gene fragment of nearly 820 bp (Simon et al., 1994). A 25 µl reaction mixture comprising 12.5 µl of Ready to use PCR master mix (Promega M750A), 5.5 µl of nuclease free water, 1 µl each of forward and reverse primer, and 5 µl of DNA template was used for PCR amplification. A 3 µl amplified PCR product was run for 45 min on a 1.2% agarose gel in 1X TAE at 100V (Jordan Scientific). The amplified PCR products were sent to AgriGenome (Kochi, India) for purification and sequencing.

All whitefly sequences from the study were aligned using ClustalW, which was implemented in BioEdit v7.2.5, before being analyzed with Mega X for phylogeny (Kimura 1980; Kumar et al., 2018). The phylogenetic tree was constructed using maximum likelihood approach and the Kimura 2-parameter model. A bootstrap replication of 1000 was run to test the phylogenies (Felsenstein, 1985). The whitefly sequences were assigned to species based on pair-wise sequence divergence greater than 3.5% upon clade formation (Dinsdale et al., 2010). All the whitefly sequences were submitted to the NCBI GenBank to obtain accession numbers. DnaSP v5.10 was used to define the sequence polymorphism, singleton variable sites, average nucleotide differences, G+C content, and number of haplotypes, haplotype diversity and nucleotide diversity of the *B. tabaci* COI sequences (Librado and Rozas, 2009). The popART software was used to analyze the haplotype network of *B. tabaci* sequences by constructing a minimum spanning network relationship among the cryptic species.

RESULTS AND DISCUSSION

Based on the maximum likelihood approach, the phylogenetic analysis of 13 samples from different agro climatic regions showed a total of 4 different mitotypes namely Asia 1, Asia II 1, Asia II 5 and Asia II 7. Asia II 1 was found prevalent and distributed in 6 out of thirteen regions viz., Banswara: Rajasthan, Sri Ganganagar: Rajasthan, Amravati: Maharashtra, Hisar: Haryana, Indore: Madhya Pradesh and Bathinda: Punjab. Asia 1 mitotype detected in three agroclimatic regions namely Meerut: Uttar Pradesh, Guntur: Andhra Pradesh and Raipur: Chhattisgarh. The samples from Jalandhar: Punjab and Coimbatore: Tamil Nadu identified as Asia II 5 whereas samples collected from two different farmscapes within Pusa: New Delhi were grouped as Asia II 7 (Fig. 1). Colour coded pair wise identity matrix for similarity scores of mitotypes of *B. tabaci* was carried out using Sequence Demarcation tool Version 1.2 (SDTv1.2) and was represented in Fig. 2. The distribution and frequency of different mitochondrial haplotypes of *B. tabaci* cryptic species belongs to India grouped as H1 (MN830428_Meerut Asia1; MN830436_Guntur_Asia1; MN830440_ Raipur_Asia1), H2(MN830429_Banswara_AsiaII1), H3(MN830430_Sri_Ganganagar_AsiaII1; MN830431_ Amaravati_AsiaII1; MN830432_Hisar_AsiaII1; MN830433_Indore_AsiaII1), H4(MN830437Batinda_ AsiaII1), H5(MN830434_Jalandhar_AsiaII5), H6(MN830438_Coimbatore_AsiaII5), H7(MN830435_ Delhi_AsiaII7; MN830439_New_Delhi_AsiaII7). The number of haplotypes, total number of variable sites, haplotype diversity, nucleotide diversity, average number of nucleotide differences, G+C content and total number of mutations are represented in Table 1. Nucleotide diversity observed higher in Asia II 5 (0.00388) followed by AsiaII 1 (0.00258) and in the same way, haplotype diversity observed higher in Asia II 5 (1.000) followed by Asia II 1 (0.600).

In total, seven haplotypes were identified from 13 analyzed sequences among them; Asia II 1 observed with three haplotypes, Asia II 5 had two whereas

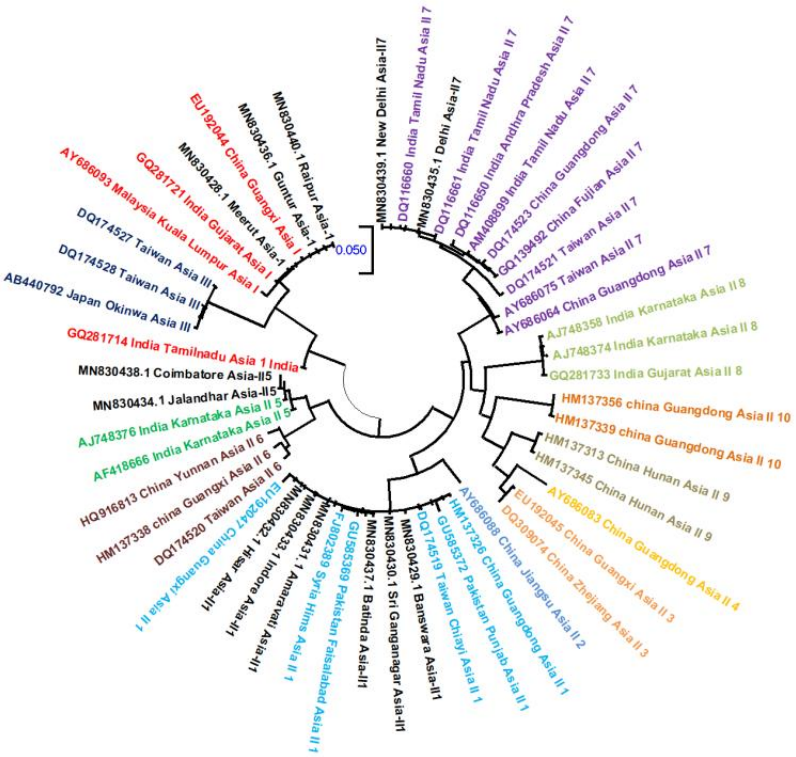

Fig. 1. Phylogenetic tree of mtCOI mitotypes of *B. tabaci* including other cryptic species as out groups using maximum likelihood Approach in MEGA X. Samples from study indicated in black colored text: all other sequences obtained from GenBank

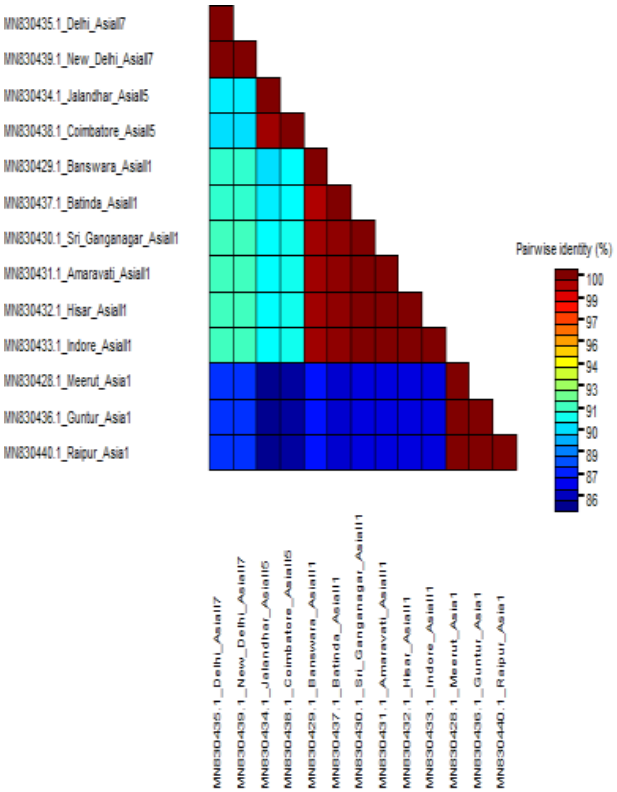

Fig. 2. Color coded pair wise identity matrix for similarity scores of mitotypes of *B. tabaci* (using Sequence Demarcation tool Version 1.2- SDTv1.2)

Asia1 and Asia II 7 had one haplotype each. The relationship among the haplotypes of each mitotype was determined by minimum spanning network analysis (Fig. 3). The four mitotypes were diversified into seven haplotypes that were highly distant from each other. Among the three haplotypes of Asia II 1, the H3 haplotype occupied the center between H2

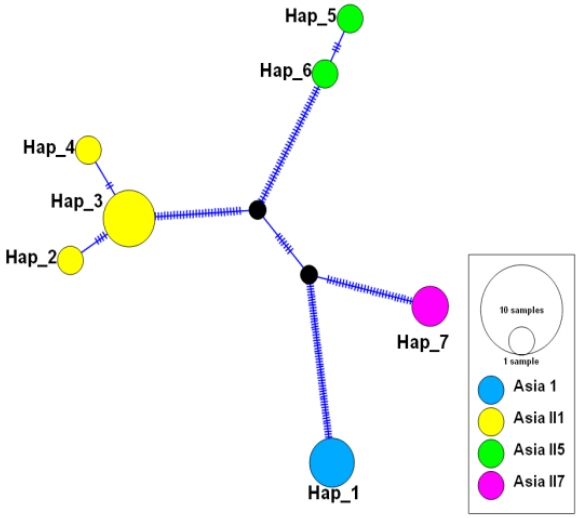

Fig. 3. Minimum spanning network from mitotypes of *B. tabaci* (using PopART-Population analysis with reticulate trees software)

Table 1. Genetic diversity analysis in mitotypes of *B. tabaci* India

Mitotypes	No. Sequences	No. haplotypes	Number of variable sites, S	Haplotype diversity	Nucleotide diversity Pi	Average number of nucleotide differences, k	G+C content	Total number of mutations, Eta:
Asia1	3	1	0	0.000	0.00000	0.000	0.316	0
AsiaII1	6	3	6	0.600	0.00258	2.000	0.339	6
AsiaII5	2	2	3	1.000	0.00388	3.000	0.348	3
AsiaII7	2	1	0	0.000	0.00000	0.000	0.335	0

and H4 in the network. Reports of mitotypes from our results coincide with previous reports in categorization of mitotypes in collected localities. Asia II 1 mitotype found in 6 out of 13 locations and proved prevalent from our study. In the same way, Asia II 1 was observed to be the most abundant mitotype with a countrywide distribution and the highest haplotype diversity, and was also found to be closely linked by its outbreak in endemic cotton leaf curl virus prone regions. One such plausible threat posed by the Asia II1 genetic group is that it has the ability to expand its range by displacing previously established mitotypes in cotton and other Agro ecosystems (Prabhulinga et al., 2020; Ashfaq et al., 2014; Ahmed et al., 2011). The presence of Asia II1 genetic group in a northern cotton-growing region and Asia I in a south-central cotton-growing region has been confirmed by maximum likelihood phylogenetic analysis, which was supported by previous reports on different whitefly host crops in India (Chowda-Reddy et al., 2012; Ellango et al., 2015).

Samples from two different farmscapes within Pusa: New Delhi location are grouped and detected Asia II7 and the results are in support by Hashmi et al. (2018) who previously reported that Asia II 1 and Asia II 7 are the leading genetic groups occurring in Delhi. The Asia II 7 cryptic species have been reported in a majority of Asian countries (Kanakala and Ghanim, 2019). It has been found in Pakistan (Ashfaq et al., 2014; Islam et al., 2018), India (Ellango et al., 2015), Indonesia (Firdaus et al., 2013), China (Qiu et al., 2011), Malaysia (Shadmany et al., 2019) and Taiwan (Shadmany et al., 2019). As on date 13 *B. tabaci* genetic groups viz., Asia I, Asia I India, Asia II 1, Asia II 5, Asia II 6, Asia II 7, Asia II 8, Asia II 11, Asia II 13, Middle East Asia Minor (MEAM) 1, MEAM K, China 3 and China 7 have been recorded from India (Rehman et al., 2021; Kanakala et al., 2019; Tamilnayagan et al., 2018; Naveen et al., 2017; Ellango et al., 2015; Singh et al., 2012; Rekha et al., 2005). The successful control of whiteflies by knowing their identity, biology and biocontrol strategies has the potential to save a million tonnes of pesticides from being used in farmer fields. Population genetics of many insect species is influenced by a variety of factors, such as environmental and ecological factors, alternative host plants, natural barriers, migration and human interference. These factors also have an impact on species diversity and distribution thus, studying diversity, distribution, and changes in population structure is essential. Similar studies should be carried out by using population genetics and phylogenetic analysis of important pests like, *B. tabaci*.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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MIRROR REPEATS IN THE *INTERSEX* GENE OF *DROSOPHILA MELANOGASTER* MEIGEN

Kavita Saini, Namrata Dangi and Vikash Bhardwaj*

Department of Zoology, Baba Mastnath University, Asthal Bohar Rohtak 124021, Haryana, India
*Email: vikashbhardwaj@gmail.com (corresponding author): ORCID ID 0000-0001-6569-665X

ABSTRACT

Genomes of many organisms, including both prokaryotes and eukaryotes, contain numerous repeat elements. Among these DNA repeats, mirror repeats have been studied rarely. Hence, this study on the mirror repeats within the *intersex* gene of *Drosophila melanogaster*, which reveal 11 mirror repeats. Out of these mirror repeats, ten mirror repeats are perfect, and six are within a single exon. The presence of mirror repeats within the intersex gene raises further questions concerning their origin, evolution, and biological function.

Key words: *Drosophila melanogaster, intersex* gene, mirror repeats, DNA repeats, parallel complement, *Drosophila* spp., origin, evolution, function

Drosophila melanogaster, also known as the fruit fly, is a model organism with widespread use in classical and modern genetics and biomedical research (Wangler et al., 2015; Mirzoyan et al., 2019; Pandey and Nichols, 2011; Tolwinski, 2017). Its quick generation time and cheap maintenance cost make it appropriate for studying extensive pathways (Mirzoyan et al., 2019). Its genome has been annotated with 17,726 genes, 13,907 of which are protein-coding genes that encode 21,953 distinct polypeptides (Kaufman 2017). Over 75% of disease-related genes have the same function in humans and flies, enabling researchers to conduct studies on flies and adapt their results to human biology (Reiter et al., 2001; Aquilina and Cauchi, 2018; Evangelakou et al., 2019). The nucleotide base sequences in the genome of any organism are methodically organized, information-rich patterns composed mainly of repeated DNA sequences; and wide variance in genome size is the consequence of repeated DNA sequences and these sequences aid in explaining genome function and structure (Hartl et al., 1994; Kapitonov and Jurka, 2003; Gurusaran et al., 2013). Repeated DNA sequences are genomic segments that repeat themselves throughout the genome and can range from a few to several thousand nucleotide sequences that are incorporated into the genomes of higher animals (Britten and Kohne, 1968; Jurka et al., 2007; Biscotti et al., 2015).

Since publishing whole-genome data from many organisms, the measurement and categorization of repeat elements have been an area of interest in computational biology research (Volfovsky et al., 2001; Treangen and Salzberg, 2012). There are several forms of repetitive elements present in the DNA of different organisms, including inverted repeats, direct repeats, tandem repeats, interspersed DNA repeats (LINES and SINES), and mirror repeats (Jurka et al., 2007; Treangen and Salzberg, 2012). To the best of our knowledge, mirror repeats have never been investigated within the genome of *D. melanogaster*. An essential objective in the study of eukaryotic developmental genetics is to understand the processes that determine sexuality. The somatic sexual differences in *D. melanogaster* are controlled by a hierarchical network of regulatory genes (Burtis and Baker, 1989; Baker, 1989; Christiansen et al., 2002; Peng et al. 2021). For the female sex differentiation, the *intersex* gene operates in conjunction with the *doublesex* gene, which is located at the very bottom of the hierarchy of sex determination(Acharyya and Chatterjee 2002; Garrett-Engele et al. 2002a; Garrett-Engele et al. 2002b; Cavaliere et al. 2009; Arunkumar and Nagaraju 2011). Consequently, the current study uses a manual computational approach to locate mirror repeats within the *intersex* gene that play a role in sex determination in *D. melanogaster*.

MATERIALS AND METHODS

The whole gene sequence for the *intersex* gene (Gene ID 45881) was retrieved from the NCBI and saved in the FASTA format. Further, the sequence was split, generating two distinct sequences consisting of 500 and 227 base pairs, respectively (the total number of base pairs in the *intersex* gene is 727). A parallel complement sequence of each component

was retrieved using the Reverse Complement tool, which can be found at https://www.bioinformatics.org/sms/rev comp.html. The original sequence and its parallel complement sequence were aligned for homology searches using the BLAST software, which may be found at https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE TYPE=BlastSearch&LINK LOC=blasthome. The program selection was changed to a nucleotide sequence pairwise, and the word size was set to 7. Both sequences were initially aligned at different threshold values, as shown in Table 1. The instances where the number of hits was maximum were selected, and then searched for mirror repeats in each hit. The hits were examined, and where the nucleotide position number was precisely reversed in both the subject and the query sequences were marked as mirror repeats. As shown in Table 2, the detected mirror repeats were divided into two categories: perfect and imperfect. Identified mirror repeats were also explored in the genomes of two other species, *D. nasuta and D. bipectinata*. Fig. 1 explains the complete methodology that is used in this study.

RESULTS AND DISCUSSION

The identification of mirror repeats in the *intersex* gene of *D. melanogaster* was performed using a manual bioinformatics computational approach. Only one exon is included in the *intersex* gene, with a total sequence length of 727 base pairs. It was observed that the number of hits also increases with increasing E value, and once the E value reaches a certain threshold, the number of hits saturates and stops increasing further. Mirror repeats were also searched in all hits, as indicated in Table 1, and when the number of hits stops going further after a specific E value, it was fixed as threshold E value. After going through this procedure, 11 mirror repeats were retrieved in the complete gene sequence. The gene was split into two parts and found the mirror repeats on the individual segment. The same number of mirror repeats were found in both cases, and there are 11 mirror repeats in the entire gene, and there are 11 mirror repeats cumulatively in both parts. This led to the conclusion that the number of mirror repeats extracted is the same in both cases. Considering the findings presented in Table 1, 20 such repeats were selected, the

Table 1. No. of mirror repeats in exon, gene parts and complete gene at different E values

Expected threshold (E value)	No. of hits for Exon	Mirror repeats in Exon	No. of hits for Part-1 1-500	Mirror repeats in Part-1 1-500	No.of hits for Part-2 501-727	Mirror repeats in Part-2 501-727	No. of hits for complete sequence	Complete gene
0.05	0	0	0	0	1	1	0	0
10	16	3	13	3	8	6	27	8
15	16	3	13	3	8	6	27	8
20	29	6	27	5	8	6	27	8
40	29	6	27	5	8	6	47	11
100	29	6	27	5	8	6	47	11
Total mirror repeats		6		5		6		11

Table 2. List of mirror repeats in *intersex* gene of *D. melanogaster*

S. No.	Mirror repeat gene ID- 45881	Position of mirror repeats	Type	Length
1.	AAAAGAAAA	13-21	Perfect with single spacer	9
2.	GGACCCCCAGG	151-161	Perfect with single spacer	11
3.	CCAGGACC	157-164	Perfect mirror	8
4.	TCGCGCT	305-311	Perfect with single spacer	7
5.	ATCACTA	470-476	Perfect with single spacer	7
6.	ACATACA	575-581	Perfect with single spacer	7
7.	TGATTAGT	608-615	Perfect	8
8.	TTGATAGTT	642-650	Perfect with single spacer	9
9.	TTTATTATTT	666-675	Perfect mirror	10
10.	TAAATAAAT	699-707	Perfect with single spacer	9
11.	AATAAATATTCTTAGATATAA	701-721	Imperfect mirror	21

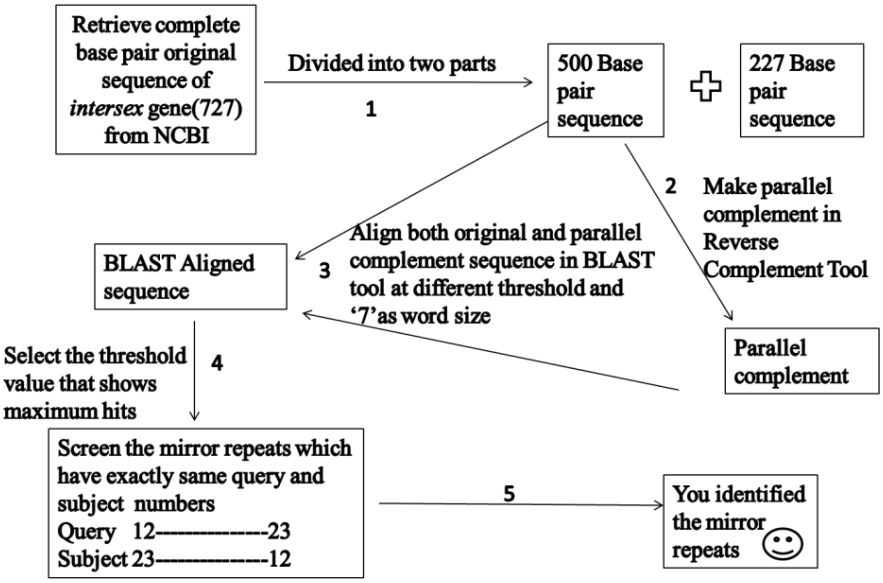

Fig. 1. Steps in extracting of mirror repeats

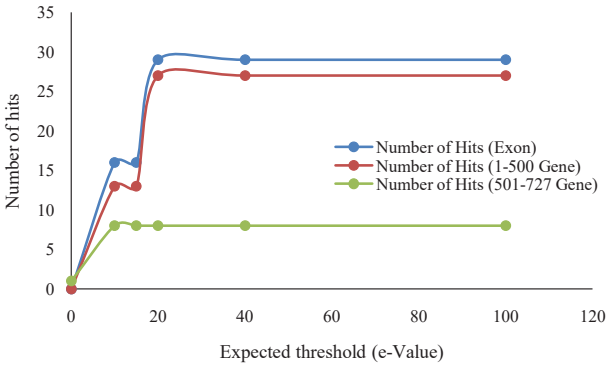

Fig. 2. Number of hits vs. expected threshold (E-value) for *intersex* gene of *D. melanogaster*

expected threshold for exons and two other sections of the gene because after this point, the number of hits and mirror repeats stops increasing further. Alternatively, 40 were selected as the expected threshold for the entire gene sequence. Here, the number of hits are directly correlated with the expected threshold shown in Fig. 2. The observation specifies that the number of hits increases as the E value increases, and once the E value reaches a definite threshold, the number of hits stops and shows a saturation`.

Table 2 demonstrates mirror repeats prevalent in the *intersex* gene of *D. melanogaster*. Subsequently, we classified mirror repeats as perfect or imperfect. Perfect mirror repeats have a 100 per cent symmetrical (CCAGGACC) arrangement of nucleotides around their centre of symmetry. Imperfect mirror repeats are not ideally 100% symmetrical (AATAAATATTCTTAGATATAA). There are 11 mirror repeats within the *intersex gene*; 3 are perfect mirror repeats without any spacer, while 7 are perfect with spacer, with only a single imperfect mirror repeat. As shown in Table 2, the *intersex* gene has many short mirror repeat sequences. This table also includes information about their location, type, and length. The mirror repeats found in the intersex gene of *D. melanogaster* range in length from 7 to 21 base pairs, with the largest repeat being 21 base pairs in length. Fig. 2 depicts the relative location of mirror repeats in the complete sequence (1-727) and two portions of the *intersex* gene. In this figure, two mirror repeats (157-164, 701-721) starts between the other two mirror repeats (151-161,699-707), overlapping each other. Table 3 highlights the mirror repeat sequences to indicate the precise location of mirror repeats within the *intersex* gene of *Drosophila melanogaster*. In this table, it is evident that two mirror repeats overlap with two other mirror repeats, but the remaining seven mirror repeats are dispersed differently.

In the *intersex* gene of *D. melanogaster*, there is just one exon. Exon locations in the *intersex* gene range is from 79 to 645 base pair sequence. There are 6 mirror repeats out of total 11 mirror repeats found in the gene's exon. 4 mirror repeats identified in an exon are perfect with single spacer mirror repeats and 2 are perfect mirror repeats and all with a maximum sequence length of 11 base pairs and a minimum sequence length of 7 base pairs. Table 4 shows the mirror repeats found in the exon of the *intersex* gene. Fig. 3 and 4 depicts the relative location of mirror repeats throughout the *intersex* gene. Table 5 highlights the mirror repeats identified

Table 3. Distribution and sequence of mirror repeats in *intersex* gene of *D. melanogaster*

CDS	Number of mirror repeats	Sequence with highlighted mirror repeats
1-727	11	TTTTTCTTCACAAAAAGAAAACAATTCGCGGCTGTTCAATATTTTT CTCCCCCAATATCCATCGATTTCGAGTGCCAAATGAATCCCAACA TGAACATGATGCCCATGTCTGGGCCACAAATGATGCAGGTAATGC AATCCTCGCCATCGGGACCCCCAGGACCAGTGCAGCATCAACAGC AGCAGCCTCCACAGCCACTGCAGCAGCAGCAGCAGGCCGAAAAA TTGGACAACATTTCCAGGGTGAAGAGTTTGCTGGGACCACTGCGG GAGTCCATGTTCCTCACCATCCGGTCGAGCGCCTTCGCGCTGCAG CAAAACAATCTCGCGGACAACTTAAAGAGGGACACGGGTGCCCA CCATGTTCCGCGGTTCGACAAGCACTTGGAAGACTTTTACGCCTG TTGCGACCAGATCGAGATCCACTTGAAGACGGCGATGCAGTGCCT CCAGCAGCAGAACTCCTCCAATCACTATCTCCCCGGTCCGGTGAC TCCCATGCGCATGGAGACCTTTATGCCGGACAACGCCGGCCCCAT TTCGTATCCCACTTACTTGAACACGGTCCGCGTTCACATACAGTCC GCCAAGGATATACACGACACTCTGATTAGTGCCGCGCAGAACATT TCGCAGGCTGATTGATAGTTGTAGTAGCATTAGGTTTTATTATTTC ACACGCATGCACTTAAGTTAAGTAAATAAATATTCTTAGATATAA GATAAC

Table 4. Shows mirror repeats in exon of *intersex* gene of *D. melanogaster*

EXON	Expected threshold	No. of mirror repeats	Mirror repeat	Position in exon	Type
79-645	20	6	1. GACCCCCAGG	73-83	Perfect with single spacer
			2. CCAGGACC	79-86	Perfect mirror
			3. TCGCGCT	227-233	Perfect with single spacer
			4. ATCACTA	392-398	Perfect with single spacer
			5. ACATACA	497-503	Perfect with single spacer
			6. TGATTAGT	530-537	Perfect mirror

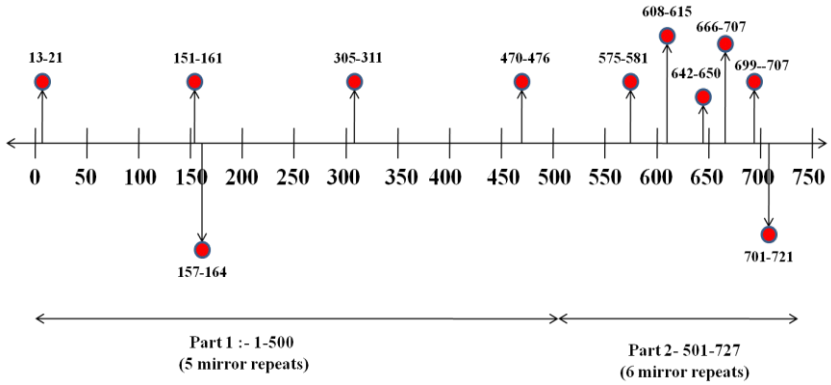

Fig. 3. Relative position of mirror repeats in CDS-1-727 *intersex* gene of *D. mealnogaster*

within the *intersex* gene's exon. This allowed us to observe that only one-mirror repeat overlaps with the another, and the remaining mirror repeats are scattered separately. Comparative observations of mirror repeats in genomes of *Drosophila* spp. (*D. nasuta* and *D. bipectinate*) through mega BLAST tool are given in Table 6; *D. nasuta* Genome possessed 9 mirror repeats in the *intersex* gene, but *D. bipectinata* had just 5. Due to their evolutionary conservation, several mirror repeat sequences identified in both *D.nasuta and D.bipectinata* may play a significant role in these genomes. The mirror repeats denoted by the – sign (indicates absence within that genome) are due to the limitations of the BLAST server tool in handling short input sequences.

Table 5. Distribution of mirror repeats with sequence in exon of *intersex* gene.

EXON	No. of mirror repeats	Sequence with highlighted mirror repeats
Exon 1	6	ATGAATCCCAACATGAACATGATGCCCATGTCTGGGCCACAA ATGATGCAGGTAATGCAATCCTCGCCATCGGGACCCCCAGGA CCAGTGCAGCATCAACAGCAGCAGCCTCCACAGCCACTGCAG CAGCAGCAGCAGGCCGAAAAATTGGACAACATTTCCAGGGT GAAGAGTTTGCTGGGACCACTGCGGGAGTCCATGTTCCTCAC CATCCGGTCGAGCGCCTTCGCGCTGCAGCAAAACAATCTCGC GGACAACTTAAAGAGGGACACGGGTGCCCACCATGTTCCGCG GTTCGACAAGCACTTGGAAGACTTTTACGCCTGTTGCGACCA GATCGAGATCCACTTGAAGACGGCGATGCAGTGCCTCCAGCA GCAGAACTCCTCCAATCACTATCTCCCCGGTCCGGTGACTCCC ATGCGCATGGAGACCTTTATGCCGGACAACGCCGGCCCCATT TCGTATCCCACTTACTTGAACACGGTCCGCGTTCACATACAGT CCGCCAAGGATATACACGACACTCTGATTAGTGCCGCGCAGA ACATTTCGCAGGCTGATTGA

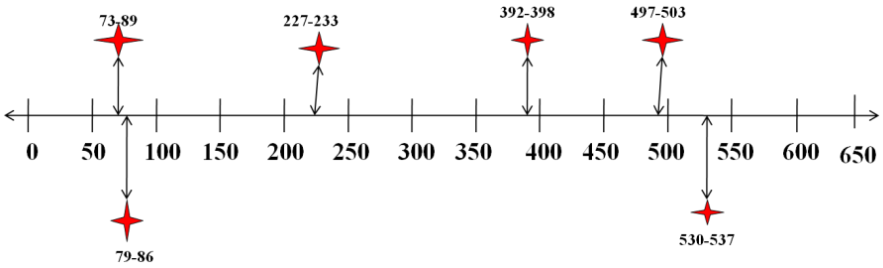

Fig. 4. Relative position of mirror repeats in CDS-73-645 *intersex* gene of *D. mealnogaster*

Table 6. Comparative analysis of mirror repeats of the *intersex* gene of *D. melanogaster* with other *Drosophila* species (*D. nasuta* and *D. bipectinata*) using mega BLAST

S. No.	Mirror repeats	Mirror repeats in *intersex* gene of *D. melanogaster*	Genome of *D. melanogaster*	Genome of *D. nasuta*	Genome of *D. bipectinata*
1.	GGACCCCCAGG	+	+	-	+
2.	AAAAGAAAA	+	-	+	+
3.	CCAGGACC	+	-	+	-
4.	TCGCGCT	+	-	+	-
5.	ATCACTA	+	-	+	-
6.	AATAAATATTCTTAGATATAA	+	+	-	-
7.	TTTATTATTT	+	-	+	+
8.	TTGATAGTT	+	-	+	+
9.	TAAATAAAT	+	-	+	+
10.	TGATTAGT	+	-	+	-
11.	ACATACA	+	-	+	-

This study applied a manual computational approach to identify mirror repeats with*in the intersex* gene of *D. melanogaster*. A single gene may perform different functions in different cellular environments. Moreover, a gene may be found in multiple species that perform either the same or different functions. This study observed the presence of mirror repeats in the exon and gene sequences of an *intersex* gene. A total of 11 mirror repeats were identified in the complete gene sequence and out of which 6 mirror repeats are present in the exon of this gene. It will be highly significant to find the role of mirror repeats at the molecular level concerning transcription and translation processes within the cell. However, to date, the exact function of mirror repeats has not been elucidated. Further studies may be required to determine the exact role of mirror repeats.

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AUTHOR CONTRIBUTION STATEMENT

KS and VB formulated the study, KS performed all the bioinformatics analysis, KS and ND drafted the manuscript, and VB supervised the project and reviewed the manuscript.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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ECOLOGICAL ROLE OF *ONTHOPHAGUS TAURUS* (SCHREBER) IN SOIL NUTRIENT MOBILIZATION

Harshita Johari, Nishi Pandya, Pankaj Sharma and Pragna Parikh*

Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, Gujarat, India
*Email: php59@yahoo.co.in (corresponding author): ORCID ID 0000-0001-7990-3517

ABSTRACT

Dung beetles play a major role in the pasture ecosystem. The manure recycling activity of dung beetles is linked to their tunneling behavior. The present study was designed to analyze the tunnel pattern and nutrient mobilization by dung beetles, *Onthophagus taurus* (Schereber, 1759) in different soil types. A simple type of tunnel pattern was observed in all the four types of soil after 30th day of their introduction (10 pairs of male and female) into the experimental setup. However, the maximum number of tunnels was observed in the sandy and sandy clay loam (no. of openings- 15), followed by loamy soil (no. of openings- 13). The physical (texture, water holding capacity, porosity, moisture content) and chemical parameters (pH and nutrients) of all the four types of soils were evaluated. Soil texture analysis revealed the texture to be of sandy (yellow soil), sandy clay loam (red and black soil), and loamy sand (brown soil) types. Water holding capacity and the soil porosity were recorded highest in the sandy soil, whereas moisture content was found maximum in the sandy clay loam. Soil nutrient analysis illustrated a significant increase in the amount of nitrogen (N), phosphorus (P), calcium (Ca), sulfur (S), sodium (Na), potassium (K), organic carbon and organic matter. Thus, the present study confirms that tunneling activity of *O. taurus* enhances the soil nutrients by carrying out dung decomposition.

Key words: Dung beetle, *Onthophagus taurus*, nesting, tunneling, nutrients, soil parameters, texture, water holding capacity, porosity, moisture, nutrients, sandy, clay, loam, red and black soils

Arthropods are one of the most successful and cosmopolitan group of animals on earth. Their ability to adapt to the changing environment makes them the most successful and diverse group of animals (Giribet, 2019). Among the arthropods, class Insecta is the largest group and the order Coleoptera is the leading order of the animal kingdom constituting almost 25% of all the living organisms and it includes around 3,50,000 species worldwide and among these around 15,088 species are present in India. Among 25% of insect species, 40% are beetles (Thakkar, 2016). Scarab beetles commonly known as dung beetles of the family Scarabaeidae have approximately 30,000 species of beetles (Cajaiba et al*.,* 2017). They exhibit a wide range of ecological, morphological as well as behavioral adaptations which makes them universally distributed. Mostly dung beetles prefer to be omnivore, than herbivore dung, and the least preferred is carnivore dung (Frank et al., 2017, unpublished data). Mandibles and maxillae of adult dung beetles have a fine outer edge which helps in modifying and filtering out the content of dung (Shukla et al*.,* 2016). Further, tibia of forelegs have spines and spurs which helps them in digging and forming the tunnel. Tibial spur number varies among the species which helps taxonomist to classify the dung beetles (Linz et al*.,* 2019). In addition, head of the dung beetles has a hard, scoop like structure which helps in rolling the dung balls for their nesting (Ix-Balam et al*.,* 2018). *Onthophagus taurus* (Schreber), as a tunneler makes "multimedia galleries" (tunnels) deep into the soil for laying eggs in the brood balls. These tunnels can be formed by both male and female or only by single parent. Brood balls are placed into the blind end of the tunnel. Single branch of these complex tunnels may contain one or multiple brood balls (Tonelli, 2021). This behavioral aspect enhances their ecological efficiency for dung decomposition, bioturbation, seed dispersal, parasite suppression, fly control and nutrient recycling (Shahabuddin et al., 2017). Further, tunneling activity makes the continuous movement of the soil and thereby increases soil aeration and its water holding capacity (Nichols et al*.,* 2008; Doube, 2018). Dung produced by livestock are source of many greenhouse gases such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) which is reduced by dung beetles by reducing organic matter from the dung by their relocation into the soil (Piccini et al*.,* 2017).

There are generally four types of soil present in nature; sandy, clay, silt and loamy with varying size and texture. Reviews suggest that Vadodara, Gujarat has different types of soil which includes clay, clayey sand, gravel, sand, silt and silt sand (Sabhaya et al., 2018). Although the dung beetles play an important part in agroecosystem, no study has been conducted on the role of dung beetles in the nutrient mobilization in varying soil types of Vadodara district. *O. taurus* is widely distributed in many parts of the country (Sabu et al., 2011; Chandra et al., 2012; Pawara et al., 2012; Gupta et al., 2014; Thakkar and Parikh, 2016), including Vadodara (Singhal et al., 2018) city of Gujarat, making them a suitable model for the study. Hence, in the present work, an attempt is made to elucidate the behavioral and ecological role of *O. taurus* in soil nutrient mobilization.

MATERIALS AND METHODS

Onthophagus taurus along with the surrounding soil and dung was collected by hand pick method from Channi (22.363˚N, 73.166˚E), Kelanpur (22.241˚N, 73.269˚E), Sindhrot (22.331˚N, 73.063˚E), and Timbi (23.149˚7N, 74.002˚E) of Vadodara district. The collection was done during the dawn and dusk, from June to November, during 2021-2022. Identification of the species was done using standard taxonomic keys (Arrow, 1931; Balthasar, 1963; Chandra and Gupta, 2013) and comparing it with the specimens in laboratory, in the Department of Zoology. Four soils collected were sandy (yellow), sandy clay loam (red and black) and loamy sand (brown). Initial identification of soil was done based on colour which was further confirmed from texture analysis using standard guide (Maiti, 2003). The experimental setup was maintained in the laboratory condition where earthen pots (40 cm high, 20 l) were used half filled with yellow, red, black and brown soil and with release of 10 pairs (male and female) in each pot. Laboratory conditions (28ºC, 70% RH, 10:12 hr light regime were maintained. Soil conditions were checked at regular interval using the hygrometer (HTC-2, India) (Pandya et al., 2022). Fresh dung of buffalo was collected with the help of showel from the same site of beetle's collection and a 250 g was provided at regular intervals. The pots were covered with muslin cloth and the setup was maintained for 30 days to observe the tunnels. The dung beetle's rate of reproduction, brood ball formation and tunneling activity were observed to be increases from 20 days onwards with maximum being after 30 days (Pandya et al., 2022).

Therefore, tunnel pattern was monitored by studying its lifecycle and the number of brood balls formed at the end of 30th day, and brood morphometry of each developing stage were recorded. At the end of 30th day, tunnels were filled with the plaster of Paris up to the opening and were allowed to solidify. After 24 hr, the solidified tunnels were excavated carefully from the soil and dimensions of the cast were measured following Sinha (2014). First, the physical parameters (soil texture, water holding capacity, moisture content and porosity) as well as chemical parameters (pH) of all four soils were analyzed (Saxena, 2001). Next, to check the impact of soil type in tunnel formation as well as to see the ecological role of dung beetle in nutrient cycling, the soil nutrient analysis was done for all soil types and two groups viz., I: (control): Soil + dung beetles; and II: (experimental): soil + dung beetles+ dung were maintained. A total of eight soil samples were collected on 30th day, oven dried and homogenized, then, the nutrients, organic carbon and organic matter were analyzed following Maiti (2003). Statistical analysis was done using the Graphpad Prism software, version 8.4. Two-way ANOVA was used to compare groups followed by multiple comparison test to test the significant differences ($p \leq 0.05$).

RESULTS AND DISCUSSION

Identification of the soil was done based on the colour and was identified as yellow, red, black, and brown soil. Collected *O. taurus* showed unique characters and distinct sexual dimorphism, where males possessed a pair of horns while its females encompassed a broad, hardened plate like structure on their head (Fig. 1A). Both male and female clearly depicted teeth like structures on front tibia called as tibial teeth utilized in digging (Chandra and Gupta, 2013). Being a holometabolic insect, egg, larva, pupa and adult were observed and lasted 90-120 days (Fig. 1B). Brood development period and morphometrics were analysed (Table 1); development period was observed to be 12-16 weeks, and the size of the brood ball was also normal. Barkhouse and Ridsdill-Smith (1986) and Kaur et al. (2021) on *Onthophagus binoidis*, *O. coenobitis*, *O. fracticonis* and *O. vacca* observed that the soil type as well as the moisture content affects the larval development. Among the four types of the soil, the most preferred was yellow soil (sandy, 4% moisture) followed by red soil (sandy clay loam, 6% moisture) and brown soil (loamy sand, 3% moisture), while the least preferred was black soil (sandy loam soil, 11% moisture) (Table 3). Maximum brood balls

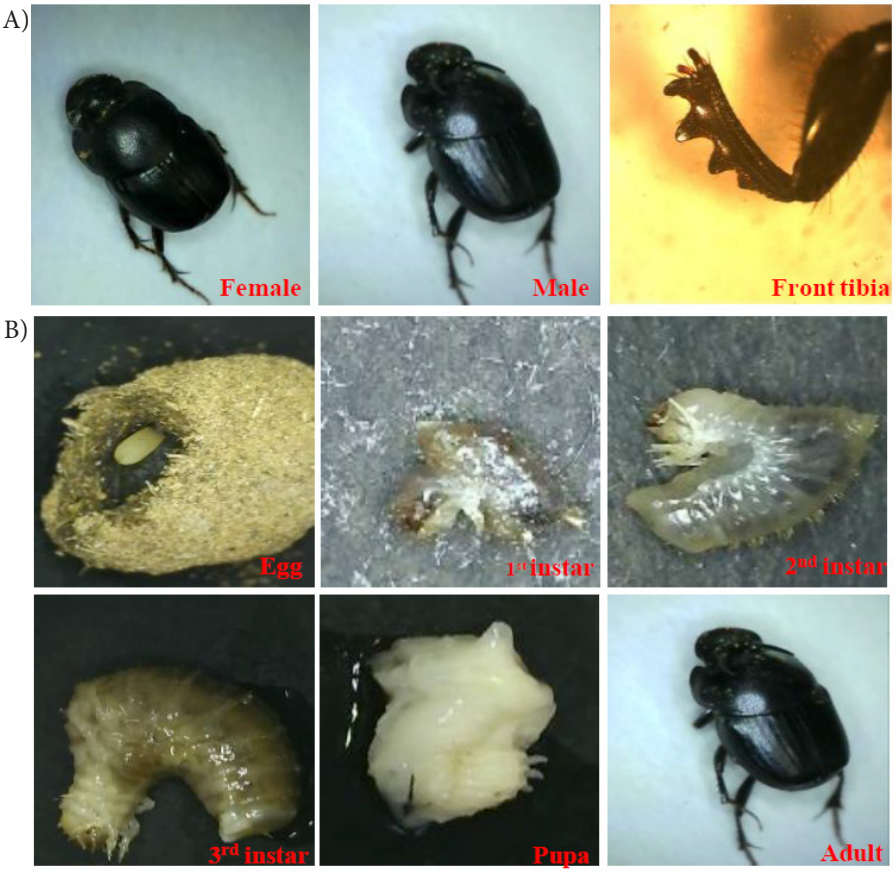

Fig. 1. Morphological characters of *O. taurus*. A. from right side- Female (no horns on head); Male (with horns on head); and front tibia with teeth (used for digging the soil and to make tunnels). B. Lifecycle of *O. taurus*- egg, larva-1st instar, 2nd instar, 3rd instar, pupa and adult (here, male)

Table 1. Developmental stages and brood morphometry of *O. taurus*

	Minimum days	Maximum days	Length (cm)	Width (cm)	Weight (g) (without brood ball)
Egg	2	4	0.1	0.1	0.01
1st Instar	2	3	0.4	0.3	0.014
2nd Instar	3	6	0.6	0.4	0.022
3rd Instar	4	8	0.9	0.5	0.030
Pupa	6	9	0.6	0.4	0.032
Adult male	80	90	1.2	0.9	0.056
Adult female	80	90	1.0	0.6	0.034

were observed in red soil (sandy clay loam- 22). *O. taurus* has ability to bury large amount of dung with a significant tunneling behaviour, and tunnel pattern in all soil types were analyzed (Table 2); and maximum area covered was in the yellow soil followed by brown soil, red and black soil (Fig. 2). Warren and Taylor (2014) observed that the tunneling is dependent on the compaction of soil, and has suggested that loam, silt loam, sand loamy to be the most suitable. Soil texture, water holding capacity, moisture content and soil porosity were analyzed (Table 3); texture analysis revealed that yellow soil was sandy type, red and black soil was sandy clay loam type where as brown soil was reported to be of loamy sand type. Water holding capacity was observed to be highest in sandy soil type, while moisture content was found to be maximum in sandy clay loam soil, with porosity being high in sandy and loamy sand soil.

Table 2. Dimensions of the tunnels formed by *O. taurus*

Soil type	NO	L	TD	W	D	Area	NB	Pattern
Yellow soil	15	10	7.8	0.9	3.8	7.06	2	Simple
Red soil	15	6.9	6.9	1.1	3.9	5.95	1	Simple
Black soil	8	3.5	5.4	1.9	3.5	5.22	1	Simple
Brown soil	13	4.9	15.5	1.7	3.8	6.53	1	Simple

NO = No. of burrow opening; L= length (cm); TD= Total depth (cm);
D= diameter area (cm2); NB= no. of branches

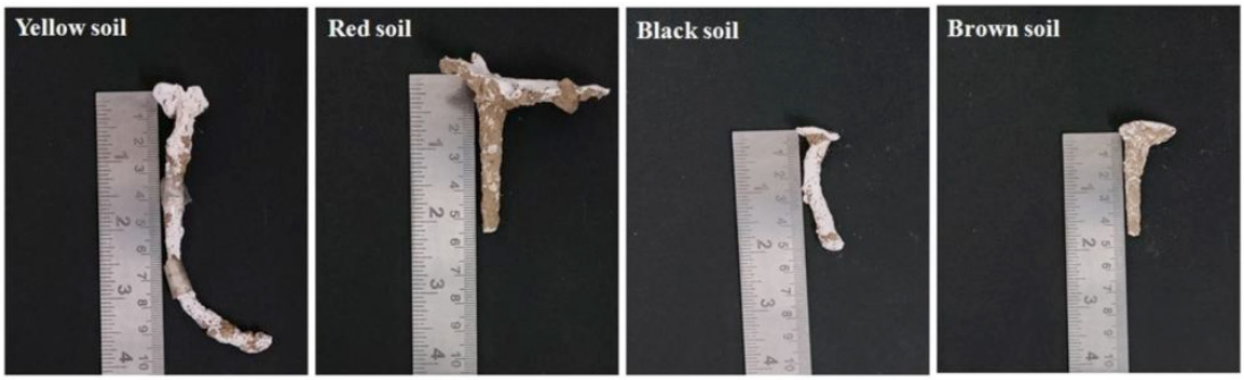

Fig. 2. Tunnel pattern in soil types after 30 days of introduction of *O. taurus*

Table 3. Texture, water holding capacity, moisture content and porosity of soil types

Soil type	Total depth (cm)	Sandy layer (cm)	Silt layer (cm)	Clay layer (cm)	% Sand	% Silt	% Clay	Texture	Water holding capacity (%)	Moisture content (%)	Porosity (%)
Yellow	11.6	11.4	-	0.2	95.27	-	1.72	Sand	11.11	4.65	100
Red	14	11	-	3	78.57	-	21.42	Sandy clay loam	19.04	6.43	92
Black	19	15	-	4	79	-	21	Sandy clay loam	20.48	11.27	47
Brown	12.3	10.2	1.8	0.3	83	14.75	2.43	Loamy sand	17.6	2.99	100

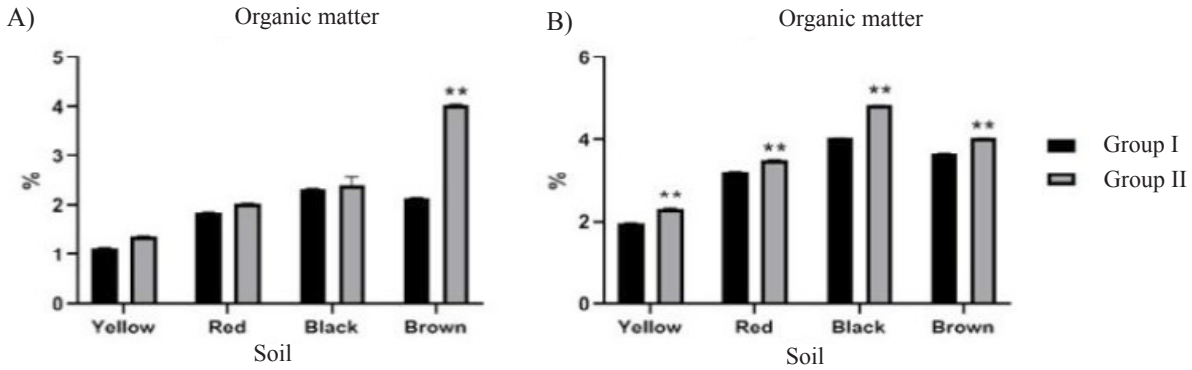

Fig. 3. Nutrient content of Soils- Group I (Control) and Group II (Experimental) after 30 days of release of *O. taurus* A. Nitrogen; B. Phosphorus; C. Calcium; D. Sodium; E. Potassium; F. Sulphur. A time dependent significant increase was observed in Group II suggesting the *O. taurus* activity- $p<0.01$**, $p<0.05$*

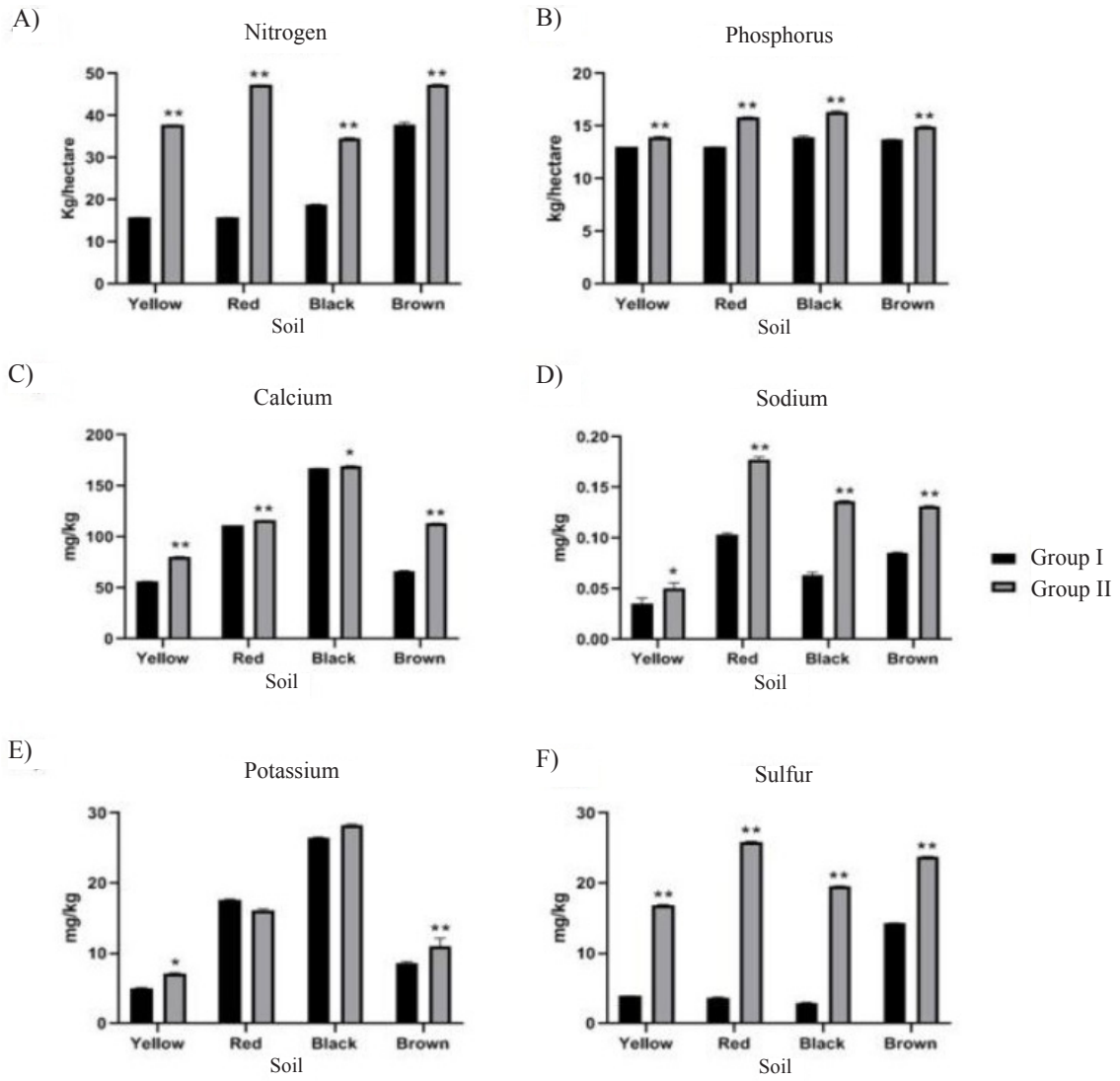

Fig. 4. A. Organic carbon, B. Organic matter- Group I (control), Group II (experimental) after 30 days of release of *O. taurus*- significant increase in the organic matter was observed after 30 days- $p<0.01$**, $p<0.05$*

Dung beetles carry out dung relocation and tunnelling activities by which it enhances soil nutrients (Bertone et al., 2006). In the present study, increase in N, K, P, Ca, Na and S as well as organic carbon and organic matter was observed in all the soil types after 30 days of release of beetles (Fig. 3, 4). Brood ball production inhibits ammonia volatilization, improving soil fertility by facilitating nitrogen absorption by plants (Maldonado et al., 2019). In addition, by making tunnels, dung beetles also help to reduce soil compaction and increase soil aeration, facilitating nitrogen mineralization (Manning et al., 2016; Xu et al., 2020; Stanbrook et al., 2021). An increase in the all the nutrients was observed in Group II in all the 4 soil types; however, N levels were found to be highest in sandy clay loam and loamy sand soilconfirming the affirmative role of the dung beetles in nutrient cycling (Zhao et al., 2008; Kronzucker, et al., 2013; Menéndez et al., 2016; Badenhorst et al., 2018; Kandil, 2019; Salomão et al., 2022). Thus, *O. taurus* appears to have an important role in nutrient cycling, and their relative importance also depends on the soil type. Their dung relocation and burrowing activities complementarily contribute to maintain soil fertility.

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AUTHOR CONTRIBUTION STATEMENT

NP and PP conceived and designed research. HS and NP carried out field visits and conducted experiments.

PS contributed gadget and analytical tool. HS and NP analyzed data. HS and NP wrote the manuscript. NP and PP revised the manuscript. All Authors read and approved the manuscript.

CONFLICT OF INTEREST/ COMPETING INTEREST

Authors have no competing interest.

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REDESCRIPTION OF SEVEN GENERA OF THE TRIBE MECYSOLOBINI (COLEOPTERA: CURCULIONIDAE: MOLYTINAE) FROM INDIA

Tarun Kumar[1 & 2]*, Guru Dayal Ram Guru[2] and Kolla Sreedevi[3]

[1]Division of Entomology, ICAR- Indian Agricultural Research Institute, New Delhi 110012, India
[2]Department of Molecular and Cellular Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj 211007, Uttar Pradesh, India
[3]Division of Germplasm Collection and Characterization, ICAR- National Bureau of Agricultural Insect Resources, Bengaluru 560024, Karnataka, India
*Email: tarunbiotech246@gmail.com (corresponding author): ORCID ID 0000- 0002- 5587- 5088)

ABSTRACT

Present study reviewed the seven genera of the weevil tribe Mecysolobini Reitter, 1913 of the subfamily Molytinae from India viz., *Brahmalcidodes* Pajni and Dhir, 1987; *Cylindralcides* Heller, 1918; *Merus* Giste1, 1857; *Neomecyslobus* Pajni and Dhir, 1987; *Ornatalcides* Heller, 1918; *Sternuchopsis* Heller, 1918 and *Tuberculomecyslobus* Pajni and Dhir, 1987. A modified key to the genera of tribe from India is also provided. All the taxonomic characters including the genitalia are illustrated, adding to the existing taxonomic knowledge of these taxa.

Key words: Molytinae, Mecysolobini, redescription, identification key, *Merus*, Oriental region.

Weevils of the tribe Mecysolobini (Coleoptera: Curculionidae: Molytinae) is mainly distributed in the Oriental region, it comprises of eight genera and more than 360 species in the oriental region (Andrew and Ramamurthy 2010). Tribe Mecysolobini with beautiful ornamentations and markings, but confused at the generic and subgeneric levels. Schoenherr (1826) described the genus *Alcides* and although Reitter described subsequent genus *Mecysolobus* in 1905, he did not consider the genus *Mecysolobus* close to *Alcides*. Heller (1918) has split the parent genus *Alcides* Schoenherr of the tribe Mecysolobini into six subgenera. Later, Heller (1933) himself expressed inadequacy of these subgenera in accommodating the known species due to less correlation variability. Marshall (1934) synonymized the parent genus *Alcides* with *Alcidodes*. Voss (1953) adopted *Alcidodes* of Marshall and divided into subgenera *Alcidodes* and *Mecysolobus*. Later, Voss (1958) substituted *Alcidodes* by *Sternuchopsis* and proposed a new genus *Mesalcidodes* between the genera *Sternuchopsis* and *Mecysolobus* and elevated subgeneric status given by Heller to the generic level.

Haaf (1961) ignored completely the classification proposed by Voss. Pajni and Dhir (1987) revised the higher taxa upon Indian species, but their work included many errors when viewed from the International Code of Zoological Nomenclature (Morimoto and Kojima 2007). Alonso- Zarazaga and Lyal (1999) listed eight genera in the Mecysolobini. Lyal and Curran (2000) proposed two species groups within *Alcidodes* for a limited number of the included species. Morimoto and Kojima (2007) prepared a key to the genera of the tribe Mecysolobini and described a new subgenus and two new species. Andrew and Ramamurthy (2010) prepared a checklist of weevils of the tribe Mecysolobini under the subfamily Molytinae, also followed the eight genera established by Alonso- Zarazaga and Lyal (1999) by covering 542 species known from Oriental and Ethiopian regions. The available descriptions of the seven genera of tribe Mecysolobini are found to be inadequate without illustrations and sufficient morphometric studies and followed the available genus name in the hierarchical list of the tribe Mecysolobini (after Alonzo- Zarazaga and Lyal, 1999 and Andrew and Ramamurthy 2010) were used So, the present study aims at redescriptions of the seven genera with key to the genera of tribe Mecysolobini. The redescriptions are supported by illustrations and morphometrics and identification key for the available genera of the tribe Mecysolobini is furnished.

MATERIALS AND METHODS

Specimens included in this study are from the NPC, Division of Entomology, ICAR- IARI, New Delhi, India. Dissections were done using a Leica EZ4 stereo zoom microscope after relaxing overnight, and

the dissected genitalia parts were placed in 10-30% KOH for 60 minutes for digestion of soft tissues. The dissected genitalia were cleaned and stored in glycerin in microvials after study pinned along with their corresponding specimens. Illustrations were made using Wild M8 Heerbrugg and Leica MZ16A stereo zoom microscopes equipped with drawing tubes and edited in Adobe Photoshop 7.0. A Leica M205 FA stereo zoom microscope with auto montage software was used to photograph the specimens. The terminology used largely follows Thompson (1992); Lyal and Curran (2000) and Kumar et al. (2016). Measurements were made using an ocular micrometer. The following abbreviations used: SL- Standard length, SW- Standard width, HL- Head length, HW- Head width, FW- Frons width, EYL- Eye length, EYW- Eye width, PL- Pronotum length, PW- Pronotum width, EL- Elytra length, EW- Elytra width, RL- Rostrum length, RW- Rostrum width, CL- Club length, CW- Club width; NPC- National Pusa Collection; IARI- Indian Agricultural Research Institute; ICAR- Indian Council of Agricultural Research; MoEF & CC- Ministry of Environment, Forests and Climate Change.

RESULTS AND DISCUSSION

Key to the Indian genera of Mecysolobini Reitter (modified after Pajni and Dhir, 1987)

1. Claw segment shallow coming out from third tarsal segment; antennal funicle robust, second to seventh segments transverse, successively becoming wider, second segment 1.28-1.32x as long as third, seventh narrower than the first of club; procoxae lying in the middle of prosternum between submarginal sulcus and posterior margin; metasternum between meso- and metacoxae longer than mesocoxa (Fig. 20) .....*Brahmalcidodes* Pajni and Dhir
- Claw segment moderately projecting out, third tarsal segment moderately bifurcated almost equal to the length.....2
2. VII funicular segment separate with the club, 0.94-0.96x narrower than I segment of club.....3
- VII funicular segment continuous from the club, 1.33-1.35x wider than I segment of club.....5
3. Funicular segment II 3.0-3.2x longer than funicular segments III to VI, 1.20-1.22x longer than segment I and VII (Figs. 4; 141), body elongated cylindrically, rostrum 1.30x moderately longer than pronotum (Fig. 1).....*Merus* Gistel
- Funicular segment II 0.9-0.92x longer than funicular segment I and VII, body elongated ovate, rostrum shallow, 1.04-1.10x as long as pronotum 4
4. Body ovate, (Fig. 49), sternite VIII of spiculum gastrale almost parallel in width (Fig. 60)..... .....*Neomecyslobus* Pajni and Dhir
- Body neither ovate nor cylindrically straight (Fig. 99), sternite VIII convex, uneven in width (Fig. 112).....*Tuberculomecyslobus* Pajni and Dhir
5. Fore- femoral tooth serrated (Fig. 37)..... .....*Cylindralcides* Heller
- Fore- femoral tooth non- serrated 6
6. Pronotum length- width ratio 0.69-0.72x as long as broad (Figs. 85; 132), elytral length- width ratio 1.33-1.42x as long as broad (Figs. 81, 139), femoral tooth 2.24x as long as broad (Fig. 153)..... .....*Sternuchopsis* Heller

   Pronotum length- width ratio 0.78-0.85x as long as broad (Figs. 68, 131), elytral length- width ratio 1.72-1.83x as long as broad (Figs. 65, 138), femoral tooth 1.0x as long as broad (Fig. 152)..... .....*Ornatalcides* Heller

RESULTS AND DISCUSSION

1. *Merus* (Gistel, 1857)

Type*: Lixus fasciatus* Redtenbacher 1848: 548. (NPC); *Merus fasciatus* Giste1, 1857: 606; *Merus fasciatus* Reitter, 1987:30.

Redescription

Body length 10.1- 12.7 mm, rostrum elongated cylindrically, with shallow punctations 1.06-1.10x widest at the apex. Antennae, slightly curved, scape elongated, straight. Eyes flat, ovate, in lateral view, covered with yellowish setae on the base. Pronotum ornamented with 3 complete yellowish longitudinal stripes, two near to the sides, one at the middle, two stripes on sides continuous to the elytra. Scutellum very small, oval shaped. Elytra elongated, straight, almost parallel in width from the shoulder to near to the apex, elytra ornamented with incomplete stripes, Abdomen U- shaped, with horizontally straight ventrites. Legs slender, foreleg longer than other legs, femur width widest at the tooth, tibia with mucro well developed, premucro absent, tarsi with claw segment well developed. Male genitalia, aedeagus almost parallel in width, narrowed, pointed at apex. Tegmen circular, manubrium, Sternite VIII divided into two hemisternites, transversely oriented. Female genitalia,

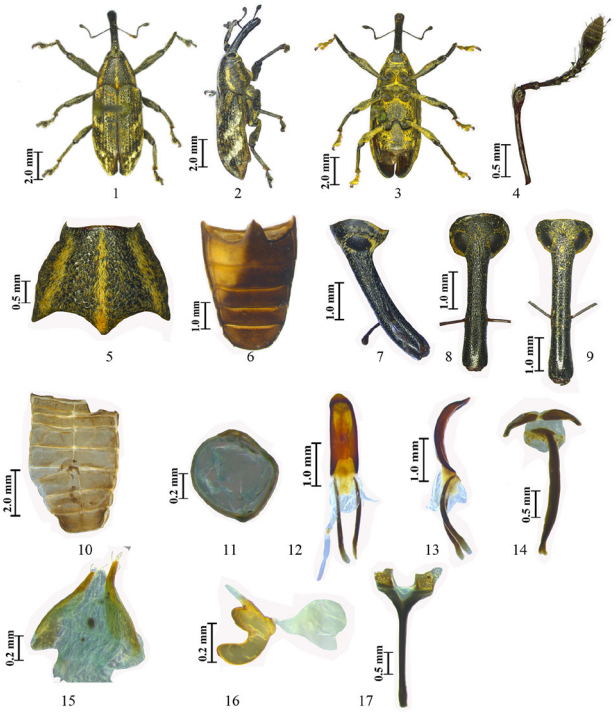
spermatheca with distal arm 1.07x as long as a proximal arm, nodulus projecting out, ramus swollen, cornu swollen, sternite VIII with spiculum ventrale, Y- shaped.

Merus fasciatus (Gistel) (Figs. 1- 17)

Type: *Lixus fasciatus* Redtenbacher, 1848: 548; (NPC); *Alcides fasciatus* Lacordiare, 1866:16; *Alcides fasciatus* Klima, 1934: 43; *Alcidodes fasciatus* Marshall, 1939: 570; *Mecysolobus fasciatus* Pajni and Dhir, 1987: 30; *Merus fasciatus* Andrew and Ramamurthy, 2010: 275.

Redescription

Head: Integument blackish with shallow punctations, densely setose; antennae reddish brown, funicles with pale yellowish white erect setae; head round at the base, 0.61-0.65x as long as broad, eyes flat ovate, 1.47-1.52x as long as broad, eyes narrow at the base of the rostrum, basal portion of the head covered with yellowish setae, frons punctated, 0.38-0.40x as wide as head (Figs. 8, 113). Antennae scape long, not reaching to the middle of the eyes, 1.17-1.20x as long as funicular segments, 8.33-8.45x as long as broad; segment I 0.80-0.85x as long as segment II, 2.00-2.14x as long as segment III to V, segment VII 1.0-1.02x as long as segment I; club 1.54-1.58x as long as broad, segment I 1.16-1.22x as long and 1.18-1.20x as broad as segment II, 1.40-1.42x as long and 2.16-2.22x as broad as segment III (Figs. 4, 141). Rostrum elongated cylindrically, shallow, punctate, apex 1.06-1.12x as wide as other portion of the rostrum, smooth at surface, 4.65-4.802x as long as broad, widest at the apex, 1.13-1.18x as wide as frons, scrobes starts near to the middle of the rostrum, almost parallel in width and curved (Figs. 7- 8; 113, 120).



Figs. 1-17. *Merus fasciatus* Gistel, Habitus (1- 3) Male dorsal, lateral and ventral view; (4) Antennae; (5) Pronotum; (6) Venter; (7- 8) Rostrum; (9) Female rostrum; (10) Sternum; Male genitalia (11- 13) Tegmen, Aedeagus dorsal and lateral view; (14) Male sternites VIII and IX; Female genitalia (15- 17) Coxites and styli, spermatheca and female sternite VIII.

Thorax: 0.79-0.85x as long as broad, with complete yellowish stripe on both sides, at the middle ornamentation with punctation, without postocular lobes, surface covered with few yellowish setae, slightly trapezoidal (Figs. 5, 127). Elytra elongated, straight, parallel in width from the base to near to the apex, with two yellowish-white stripes at the middle on each elytron, with one incomplete stripe at the base, one shallow stripe at the apex, 1.93-2.0x as long as broad, 3.00-3.15x as broad as apex, 2.90-2.95x as long and 1.18-1.20x as broad as pronotum (Figs. 1, 134). Scutellum small circular, ovate (Figs. 1, 134). Legs, foreleg longest, hindleg 0.79-0.82x as long as foreleg, femur of all three paired legs have tooth, with smaller fewer serrated denticles, femur widest at the tooth, 5.13-5.30x as long as broad, tibia well developed, tibia widest at uncus, 4.78-1.52x as long as broad (Figs. 3, 148). Abdomen: 1.43-1.50x as long as broad, all ventrites horizontally straight, ventrite I depressed at the middle, ventrite I 2.05-2.10x as long as II, 2.33-2.40x as long as III and IV, 1.84-1.86x as long as ventrite V, convex, ventrite V without erect setae (Fig. 6).

Male genitalia: Aedeagus 5.82-6.0x as long as broad, almost parallel in width, narrowed, pointed at apex; in lateral view, curved, pointed at the apex (Figs. 12- 13); aedeagal apodeme 0.95-0.98x as long as median lobe, median lobe almost parallel in width, widest at the middle, apex of the aedeagus sclerotised, membranous after the apex, endophallus transparent, shorter (Figs. 155, 169). Tegmen circular, 1.0-1.02x as long as broad, manubrium small (Figs. 11, 176). Sternite VIII (Fig. 14) divided into two hemisternites, transversely oriented, lamellate, pointed at the tip in both hemisternite; each hemisternite horizontally acuminate, sternite VIII, IX connected with almost transparent membrane; spiculum gastrale 12.5-13.0x as long as broad, slender, sinuate (Fig. 162). Female genitalia: Spermatheca with distal arm 1.07-1.10x as long as a proximal arm, the angle between arms acute, nodulus projecting out, ramus swollen, cornu swollen, narrowed at the apex (Fig.

16). Sternite VIII with spiculum ventrale 2.50-2.68x as long as broad, Y- shaped, sternite VIII divided into two hemisternites, square- shaped, transversally acuminate; coxites dilated from the styli, membranous; styli relatively small, swollen, shallow narrowed, apically inserted with long setae (Figs. 15, 17).

Measurements (mm)

Male: SL: 10.8- 11.20; SW: 3.30- 3.45; PL: 2.10- 2.25; PW: 2.70- 2.80; EL: 6.1- 6.40; EW: 3.30- 3.45; RL: 3.2- 3.35; RW: 0.40- 0.45; HL: 0.40- 0.45; HW: 0.90- 0.92. Female: SL: 11.50- 12.10; SW: 3.65- 3.90; PL: 2.30- 2.42; PW: 2.90- 2.95; EL: 6.35- 6.58; EW: 3.65- 3.90; RL: 3.45- 3.85; RW: 0.45- 0.52; HL: 0.45- 0.54; HW: 1.10- 1.15.

Remarks

Pronotum with 3 evident complete yellowish stripes, two on sides, one at the middle with punctuation without postocular lobes. Antennae, funicular segment II longer than other funicular segments. Legs with a femoral tooth, serrated, tibia with well- developed mucro and ventral tibial tooth.

Host plants

Urtica dioica, *Poaceae* spp. and *Viburnum* spp.

Distribution

India: Himachal Pradesh: Uttarakhand. Altitude: 1434–2015m.

Material examined

3♂, 4♀, INDIA: Himachal Pradesh, Manali, N32.263094°/; E77.188121°, 2009m, 28.VI.1977, Coll. unknown (NPC). 1♂, 2♀, Himachal Pradesh: Solan: Kasauli, N30.899947°/; E76.961974°, 1812m, 04.IV.1979, Coll. unknown (NPC). 2♂, 2♀, Uttarakhand: Mussoorie, N 30.45690°/; E78.07829°, 2015m, 28.VI.1980, Coll. unknown (NPC). 1♂, 1♀, Himachal Pradesh: Solan: Kandaghat, N30.967003°/; E77.107455°, 1434m, 29.VI.1980, Coll. unknown (NPC).

2. *Brahmalcidodes* (Pajni and Dhir)

Brahmalcidodes Pajni and Dhir, 1987: 29 (NPC).

Redescription

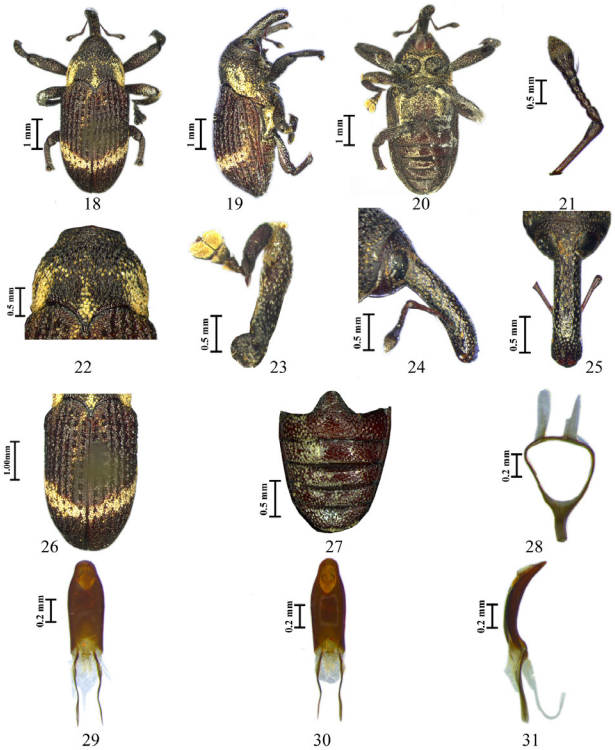
Body length 7.50- 8.0 mm. Rostrum elongated, cylindrical shaped, frons 0.49x broader than rostrum width. Eyes flat, ovate. Head with punctuations, with shallow groove. Pronotum ornamented, covered with yellowish patches on both sides, at the centre from the base to the middle, surface with irregular elongated shaped postocular lobes. Scutellum: shallow U- shaped. Elytra in dorsal view: sub- cylindrically elongated, shallow ovate, convex at the apex, almost parallel in width, elytra ornamented with a slight horizontal yellowish wide striped marking which connects at the middle of both elytra, covered with yellowish recumbent setae. Abdomen shallow, U- shaped, with horizontally straight ventrites. Legs slender, foreleg longer than other legs, femur width widest at the tooth, tibia with mucro, premucro well developed. Male genitalia aedeagus widest near to the apex of the median lobe. Tegmen with small manubrium, with long basal piece, with elongated parameres.

***Brahmalcidodes micronychus* (Pascoe) (Figs. 18- 31)**

Type: *Alcides micronychus* Pascoe, 1873: 183; Faust, 1894a: 242, 258 (NPC); *Alcidodes micronychus* Marshall, 1939: 570; *Brahmalcidodes nigromicronychus* Pajni and Dhir, 1987:30.

Redescription

Head: Integument blackish, with punctuations,



Figs. 18-31. *Brahamalcidodes micronhycus* Pajni and Dhir, Habitus (18- 20) Male dorsal, lateral and ventral view; (21) Antennae; (22) Pronotum; (23) Foreleg; (24- 25) Rostrum lateral and dorsal view; (25) Elytra; (26) Venter; Male genitalia (28- 31) Tegmen, adeagus dorsal, ventral and lateral view.

antennae dark, reddish- brown, funicles with few erect setae; rostrum apex blackish, marginate, punctate; base of head flat, 0.49-0.52x as long as broad, eyes almost round, circular; at the middle of the head in dorsal view 1.18-1.25x as long as broad; frons with shallow, punctuate depression, 0.23-0.28x as wide as head (Figs. 25, 114). Antennae scape short, not reaching to the middle of eyes, 3.79-3.85x as long as broad, smaller than funicular segments, 0.89-0.92x as long as funicles; funicle segment I 2.34-2.40x longer than funicle segment II- VI, segment I 1.80-1.85x as long as segment II, 2.31-2.40x as long as segment III- VI, segment 7 0.86-0.90x as long as segment I, funicular segment VII continuous with the club, club 1.03-1.08x as long as broad, segment I 1.56-1.60x as long and 1.38-1.42x as broad as segment II, segment II 1.30-1.36x as long and 2.20-2.28x as broad as segment III (Figs. 21, 142). Rostrum elongated cylindrically, 3.30-3.52x as long as broad, shallow, widest at the apex, shallow, punctate, marginate, with shallow longitudinal impression in the basal half, feebly curved at the middle to the apex of the rostrum; 2.06-2.12x as wide as frons (Figs. 25, 114, 121).

Thorax: integument black, filled with yellowish recumbent setae 0.86-0.90x as long as broad, dorsal view covered with punctations, shallow, dilated near to the apex, widest at the base, 1.68-1.72x as broad as apex, ornamented with yellowish patches on both sides, patches covered with setae, punctated with irregular impression (Figs. 22, 128). Scutellum small, almost oval, U- shaped (Fig. 26). Elytra shallow, ovate, 1.67-1.72x as long as broad, broadest at the shoulders, almost parallel in width from the base to near to the apex, 2.78-2.85x as broad as apex, convex shaped at the apex, with ornamentation, with shallow, horizontal, yellowish-white striped marking, connects at middle of both elytra, covered with yellowish recumbent setae; 2.07-2.20x as long as a rostrum, 2.33-2.42x as long as, 1.19-1.25x as broad as pronotum (Figs. 26, 135). Legs, fore femora slender, with a tooth, the width of the femur more at the tooth, 3.36-3.48x as long as broad, tibia well developed, with uncus 4.85-5.02x as long as broad, widest at the middle, tarsi with claw slightly coming out or somewhat rounded at the third tarsal segment into the middle (Figs. 23, 149). Abdomen: 1.28-1.40x as long as broad, all ventrites straight horizontally, ventrite continuously reduce in length from ventrite I to ventrite IV; ventrite I 2.13-2.20x as long as III; ventrite V 2.25-2.30x as long as ventrite IV, ventrite V shallow, convex; procoxae far from margin of prosternum (Fig. 27).

Male genitalia: Aedeagus 4.50-4.80x as long as broad, widest near to the apex of the median lobe (Figs. 29–31); aedeagal apodeme 0.88-0.92x as long as median lobe, median lobe uneven in width, shallow, dilated at the apex, apex of the aedeagus round, thereafter membranous; in lateral view, curved (Figs. 156, 170). Tegmen with small manubrium, with long basal piece, with elongated parameres, 2.21x as long as broad, and basal piece 1.96-2.00x as long as manubrium (Figs. 28, 177).

Measurements (mm)

Male: Paratype. Measurements (in mm): Male SL: 7.50; SW: 2.50; PL: 1.80; PW: 2.0; EL: 4.20; EW: 2.50; RL: 1.50; RW: 0.5; HL: 0.5; HW: 1.0.

Remarks

Body length: 7.5- 8.0 mm. Antennae funicular segment II- VII are robust. Pronotum with ornamentations, with shallow punctations. Elytra almost parallel in width, widest at the shoulder, with ornamentations. Legs claw segment shallow coming out from third tarsal segment.

Host plants

Viburnum spp.

Distribution

India: West Bengal.

Material examined

Paratype: 2♂, INDIA: West Bengal, Darjeeling, N26.834900°/; E88.307149°, 423m, 16.VIII.1922, Coll. Mackenzie. (NPC)

3. *Cylindralcides* (Heller)

Cylindralcides Heller, 1918- 211.

Redescription

Body length 8.00- 8.50 mm, elongated, straight. Rostrum elongated cylindrically, shallow, marginate without punctations at the apex. Antennae: scape almost reaches to the middle of the eyes. Head with a central groove at frons, eyes flat, ovate. Pronotum ornamented, covered with pale yellowish markings which cover on both sides. Scutellum triangular-shaped, emarginated at the U- shaped elytral base. Elytra in dorsal view sub- cylindrically elongated, ovate at the apex, almost parallel in width from the shoulder to near to the apex, ornamented with creamy

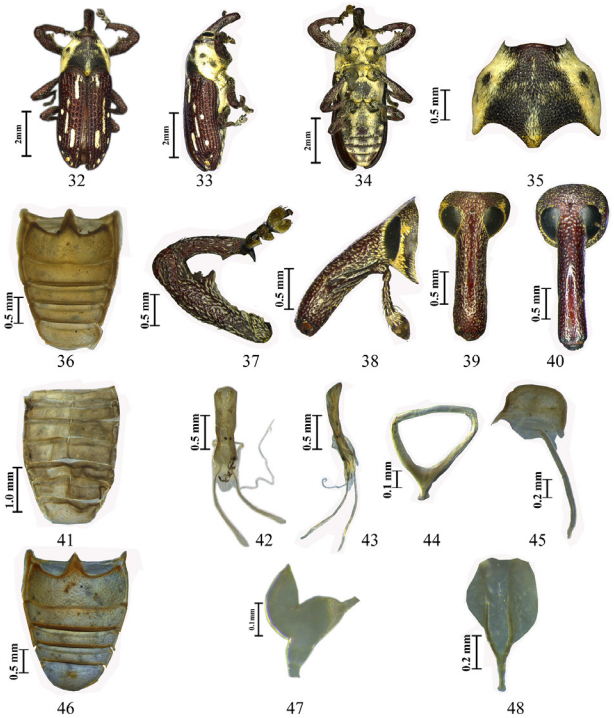
yellowish incomplete stripe distantly distributed at each interstrial interval. Abdomen almost V- shaped, covered with pale yellowish setae with reddish integument, with horizontally straight ventrites. Legs slender, foreleg longer than other legs, tibia with mucro well developed. Male genitalia aedeagus irregular in width, abruptly narrowed at the apex. Tegmen shallow, triangular shaped. Sternite VIII convex shaped. Female genitalia spermatheca the angle between distal and proximal arms acute, nodulus projecting out, and ramus flatten, cornu shallow, swollen. Sternite VIII with spiculum ventrale Y- shaped.

Cylindralcides bubo (Fabricius) (Figs. 32- 48)

Type: *Cylindralcidodes bubo* Pajni and Dhir, 1987: 32; (NPC); *Rhynchaenus bubo* Fabricius, 1801: 474; *Rhynchaenus ferox* Olivier, l807:189; Klima, 1934:52; *Alcides bubo* Boheman in Schoenherr, 1836: 612; *Alcidodes bubo* Marshall, 1939: 570; *Cylindralcides bubo* Andrew and Ramamurthy, 2010: 273.

Redescription

Head: Reddish-brown, with few recumbent pale yellowish setae surrounded on sides of the eyes; rostrum reddish-brown; head round, with a small central groove, 0.73-0.80x as long as broad; eyes flat round shaped, reaches to the base of the rostrum, sides covered with yellowish markings, 1.43-1.52x as long as broad; frons 0.33-0.38x as wide as head (Figs. 39, 115). Antennae scape almost reaches to the middle of eyes, 4.60-4.70x as long as broad, 0.88-0.92x as long as funicles; segment I 1.13-1.20x as long as segment II, 2.18-2.32x as long as segment III to IV, segment VII 1.13-1.20x as long as segment I; club 1.22-1.30x as long as broad, segment I 0.37-0.40x as long and 1.14-1.20x as broad as segment II, 0.41-0.50x as long and 1.58-1.62x as broad as segment III (Figs. 38, 143). Rostrum elongated cylindrically, shallow, irregularly marginated, 2.76-2.80x as long as broad, almost parallel in width at base and apex, 1.42-1.48x as wide as frons, scrobes almost parallel in width starts near to the middle of the rostrum (Figs. 38-39).



Figs. 32-48. *Cylindralcides bubo* Fabricius, Habitus (32- 34) Male dorsal, lateral and ventral view; (35) Pronotum; (36) Venter; (37) Foreleg; (38- 39) Male rostrum, lateral and dorsal view; (40) Female rostrum; (41) Sternum; Male genitalia (42- 43) Adeagus, dorsal and lateral view; (44) Tegmen; (45) Male sternites VIII and IX; (46) Female venter; Female genitalia (47- 48) Spermatheca and female sternite VIII.

Thorax: Shallow dilated near to the apex, widest at the base, with ornamentations, sides covered with yellow patches, setose with yellowish setae, 0.82-0.85x as long as broad, almost parallel in width after the apex, 1.84-1.90x as broad as apex, narrowed abruptly at the apex (Figs. 35, 129). Scutellum triangular-shaped (Fig. 32). Elytra elongated, irregularly straight, shallow sinuate at the middle, almost straight, parallel in width from base to near to the apex, with ornamentations, longitudinal stripe near to the apex in between interstriae 2 and 3 on each elytron, with longitudinal stripes at the middle of the elytra in between interstriae 4 and 5 at each elytron; with pale yellowish setae, with small round patches at interstriae 6 and 7 from the apex on each elytron, 1.88-1.92x as long as broad, 1.80-1.85x as broad as apex, 3.35-3.50x as long as, 5.0-5.20x as broad as a rostrum, 2.47-2.52x as long and 1.08-1.12x as broad as pronotum (Figs. 32, 136). Foreleg longer than other legs, covered with shallow yellowish setae, femur having a tooth with serrated teeth, femur widest at the tooth, 3.45-3.52x as long as broad; tibia 2.33-2.42x as long as broad, widest at the uncus, well developed, tibia well developed with a mucro, corbels open type (Figs. 37, 150). Abdomen elongated, V- shaped, 1.40-1.52x as long as broad, all ventrites horizontally straight, ventrite I shallow depressed at the middle, 2.0-2.10x as long as ventrite II, 2.50-2.60x as long as ventrite III 3.55-3.64x as long as ventrite IV, 2.0-2.16x as long as ventrite V shallow convex, ventrites covered with pale yellowish setae (Fig. 36).

Male genitalia: Aedeagus 6.70-7.00x as long as broad, irregular in width, sides of median lobe sinuate,

abruptly narrowed at the apex, in lateral view flat-shaped (Figs. 42–43); aedeagal apodeme 1.15-1.20x as long as median lobe, median lobe almost irregular in width, membranous after the apex, endophallus transparent, shorter than aedeagus, flagellum almost equal to the length of aedeagus (Figs. 157, 171). Tegmen shallow, triangular, 1.05-1.10x as long as broad (Figs. 44, 178). Sternite VIII (Fig. 45) convex, pointed tips at both sides; spiculum gastrale 1.83-1.90x as long as broad (Figs. 45, 164). Female genitalia: Spermatheca with distal arm, 1.12-1.18x as long as a proximal arm, the angle between arms acute, nodulus projecting out, ramus flatten, cornu shallow, swollen, pointed at the apex (Fig. 47). Sternite VIII with spiculum ventrale 3.0-3.25x as long as broad, Y- shaped, sternite VIII divided into two elongated hemisternites, longitudinally straight, transversally acuminate, membranous, 2.80-2.90x as long as broad (Figs. 47- 48).

Measurements (mm)

Male: SL: 8.0- 8.42; SW: 2.50- 2.68; PL: 1.90-1.98; PW: 2.30- 2.35; EL: 4.70- 4.95; EW: 2.50- 2.68; RL: 1.40- 1.60; RW: 0.50- 0.55; HL: 0.80- 0.88; HW: 1.10- 1.15. Female: SL: 8.75- 9.20; SW: 2.70- 2.80; PL: 2.05- 2.15; PW: 2.50- 2.55; EL: 4.90- 5.10; EW: 2.70- 2.80; RL: 1.50- 1.82; RW: 0.55- 0.60; HL: 0.85-0.90; HW: 1.80- 1.82.

Remarks

Antennal scape almost reaches to the middle of the eyes, funicle segment VII continuous with the club. Pronotum round, ornamented with pale yellowish patches on both sides. Elytra almost longitudinally straight with ornamentations, with incomplete stripes. Legs: femur with a serrated enlarged tooth, premucro well developed, open type corbels.

Host plants

Cyamopsis tetragonoloba and *Viburnum* spp.

Distribution

India: West Bengal, Uttarakhand. Altitude: 723–1418m.

Material examined

1♂, 2♀, INDIA: Karnataka: Banglore, Seddappati, N15.5845°/; E75.5231°, 886m, Coll. T.R. Subramaniam, 15.VI.1953, 20.XII.1959. 1♂, 1♀, Himachal Pradesh, Solan, Kandhaghat; N30.96700°/; E77.10713°, 1418m, 28.VI.1977. 2♀ Assam, Jeypore. N26.40738°/; E93.24926°, 723m, Coll. C.I.E (NPC), 02.V.1979.

4. *Neomecyslobus* (Pajni and Dhir) (Figs. 49- 64)

Type: *Neomecyslobus feae* Pajni and Dhir, 1987: 29. (NPC).

Redescription

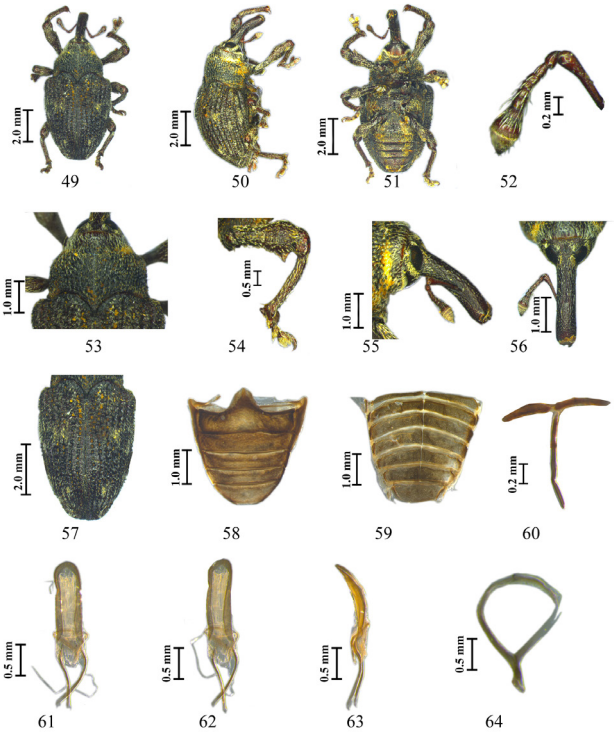
Body length 7.5- 8.0 mm, ovate. Rostrum elongated cylindrically, in dorsal view: irregular longitudinal margins visible, absent at the apex; in lateral view, scrobes almost parallel in width. Eyes flat, ovate, surrounded by yellowish setae. Pronotum without any ornamentation, shallow, covered with pale yellowish sub-recumbent setae. Scutellum very small, visible under high magnification. Elytra in dorsal view: ovate, abruptly depressed at the middle of the base, reach to the shoulder, widest at the shoulder, elytra ornamented with shallow yellowish patches. Abdomen almost U- shaped, covered with yellowish setae, with reddish integument, with horizontally straight ventrites. Legs elongated, irregular in width, foreleg longer than other legs, femur width widest at the tooth, having single pointed tooth without any serrated teeth, tibia with mucro well developed. Male genitalia aedeagus in shallow curved, weekly sclerotised. Tegmen 1.47x as long as broad. Sternite VIII divided into two hemisternites, transversely oriented.

***Neomecyslobus feae* (Faust) (Figs. 49- 64)**

Alcides feae Faust, 1894a: 245, 258; Marshall, 1939: 570; *Neomecyslobus feae* Pajni and Dhir, 1987: 31;

Redescription

Head: Blackish, with shallow punctations, with circular shallow groove at the center; antennae reddish-brown, funicles with erect setae; rostrum blackish, smooth, widest at the apex, longitudinal margin starts from the frons, reaches to the middle, with several irregular margins; base of the head flat, 0.51-0.55x as long as broad, eyes flat, ovate, 1.56-1.60x as long as broad, surrounded with yellowish setae; eyes on the middle of the head surrounded with yellowish setae; frons 0.33-0.38x as wide as head (Figs. 56, 116). Antennae scape short, not reaching to the middle of eyes, 4.37-4.50x as long as broad, 0.80-0.85x as long as funicles; segment I 0.87-0.90x as long as segment II, 1.55-1.60x as long as segment III- V, segment VII 1.42-1.52x as long as segment I, segment VII longer than funicle segments I- VI, funicle segment VII separate from the club; club 1.28-1.35x as long as broad, segment I 1.25-1.30x as long and 1.27-1.32x as broad as segment II, segment 1.0-1.10x as long as 1.83-1.90x as broad as segment III (Figs. 52, 144). Rostrum elongated



Figs. 49-64. *Neomecyslobus feae* Faust, Habitus (49- 51) Male dorsal, lateral and ventral view; (52) Antennae; (53) Pronotum; (54) Foreleg; (55- 56) Rostrum lateral and dorsal view; (57) Elytra; (58) Venter; (59) Sternum; Male genitalia (60- 63) Sternites VIII and IX, adeagus dorsal, ventral and lateral view; (64) Tegmen.

cylindrically, 3.42-3.50x as long as broad, deeply marginate with irregular margins, from the base to the middle with single longitudinal margin, emarginated from frons, reaches to the middle of the rostrum, 1.27-1.30x as wide as frons (Figs. 56, 116, 123).

Thorax: Blackish, dorsal view is covered with irregularly shaped large depressed postocular lobes, with suberect setae, without ornamentation, abruptly dilated at the apex, 0.70-0.75x as long as broad, widest at the base, 1.96-2.00x as broad as apex (Figs. 53, 130). Scutellum very small, ovate (Fig. 57). Elytra ovate, with two yellowish patches on each elytron, patch one located at the middle of the elytra starts at interstriae 3, reaches to the margin of each elytron, patch two starts at interstriae 2, reaches to the margin of each elytron, interstriae 1- 3 shallow, longitudinally sinuate, elytra shallow, truncated at the apex, 1.24-1.30x as long as broad, 3.85-3.90x as broad as apex, 1.81-1.85x as long as a rostrum, 1.85-1.92x as long, 1.05-1.10x as broad as pronotum, broadest at the base (Figs. 57, 137). Foreleg longer than the other legs, a femur having a non- serrated pointed tooth, femur widest at the tooth, 3.81-3.89x as long as broad, 5.85-5.90x as long as broad, widest at the middle, tibia well developed with a mucro, premucro shallow, visible, tibia with open type corbels, claws developed (Figs. 54, 151). Abdomen: 1.15-1.20x as long as broad, continuously narrowed from the base to the apex, ventrite I shallow, depressed, ventrite II- V straight horizontally, ventrite I 2.47-2.55x as long as ventrite II, 2.89-2.95x as long as ventrite III- IV; 1.67-1.72x as long as ventrite V, shallow convex, sternite I less in length than another sternite II- VII; procoxae far from the margin of prosternum (Fig. 58).

Male genitalia: Aedeagus 5.73-5.90x as long as broad, in lateral view shallow curved- (Figs. 61–63); aedeagal apodeme 1.20-1.25x as long as median lobe, median lobe almost parallel in width, rounded at apex, apex of the aedeagus weekly sclerotised, membranous thereafter (Figs. 158, 172). Tegmen 1.47-1.52x as long as broad, basal piece 2.0-2.10x as long as manubrium (Figs. 64, 179). Sternite (Fig. 60) VIII divided into two hemisternites, transversely oriented, each hemisternite horizontally acuminate (Fig. 165).

Measurements (mm)

Male: Male SL: 7.80; SW: 3.30; PL: 1.70; PW: 2.50; EL: 4.40; EW: 3.50; RL: 1.80; RW: 0.5; HL: 0.6; HW: 1.2.

Remarks: Antennae funicle segment VII 1.25-1.28x longer than funicular segments I and 2.23-2.30x longer than funicular segment III- VI, separate with the club. Pronotum blackish, without ornamentations. Elytra ovate, blackish, with shallow ornamentations, with yellowish patches. Legs with a femoral tooth, tibia with a well- developed mucro. Metasternum bulged towards hind coxae.

Host plants

Bidens pilosa and *Viburnum* spp.

Distribution

India: Arunachal Pradesh.

Material examined

2♂, INDIA: Arunachal Pradesh, Trista forest, N27.68917°/; E96.45972°, 1225m, 16.V.1981, 20.VI.1978. Coll. unknown (NPC).

5. *Ornatalcides* (Heller) (Figs. 65- 80)

Ornatalcides Heller, 1918: 214.

Redescription

Body length 14.0- 14.5 mm, shallow, ovate. Rostrum

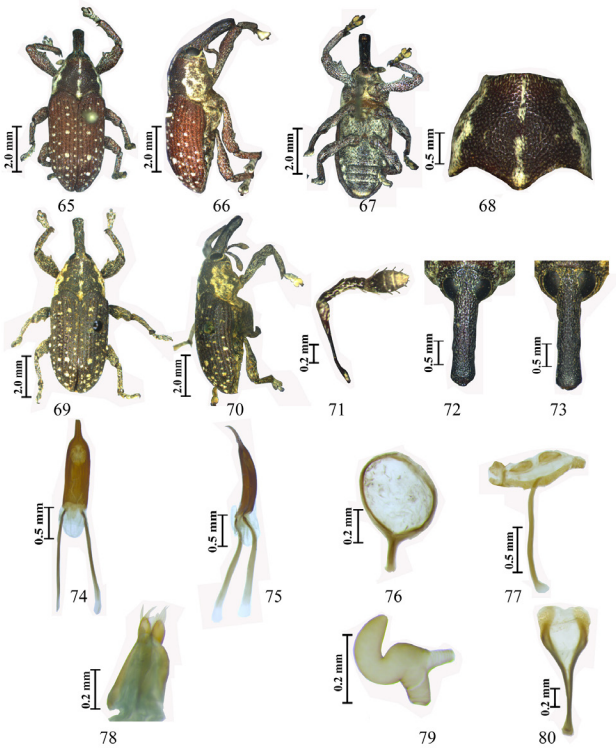
elongated cylindrically, irregular longitudinal margins visible, a small groove at the centre; in lateral view: scrobes almost parallel in width at the middle of the rostrum. Eyes flat, ovate. Pronotum ornamented, covered with creamy markings on both sides, striped at the middle with creamy setae, abruptly dilated near to the apex, oval- shaped punctations. Scutellum very small, oval reddish-brown. Elytra in dorsal view: sub-cylindrically elongated, almost parallel in width from the shoulder to near to the apex, abruptly narrowed at the apex, elytra ornamented with creamy yellowish small patches distantly distributed at each interstrial interval. Abdomen almost V- shaped, covered with pale yellowish setae, with reddish integument, with horizontally straight ventrites. Legs slender, foreleg longer than other legs, femur widest at the tooth, with a serrated tooth, claws well developed. Male genitalia aedeagus abruptly pointed apically. Tegmen circular shaped. Sternite VIII divided into two hemisternites, transversely oriented, lamellate shaped. Female genitalia spermatheca the angle between arms acute, both nodulus and ramus projecting out, V- shaped. Sternite VIII with spiculum ventrale Y- shaped, sternite VIII divided into two hemisternites, round, transversally acuminate.

Ornatalcides leopardus (Olivier) (Figs. 65- 80)

Type: *Ornatalcidodes leopardus* Pajni and Dhir, 1987: 33. (NPC); *Rhynchaenus leopardus* Olivier, 1807: 190, 296; *Alcides leopardus* Boheman in Schoenherr, 1836: 612; *Alcides leopardus* Klima, 1934: 55; *Alcidodes leopardus* Marshall, 1939: 570.

Redescription

Head: Integument blackish; rostrum apex blackish, with few recumbent pale yellowish setae near to the base; head shallow punctuate, marginate, 0.58-0.65x as long as broad, eyes flat, oval- shaped, 1.80-1.90x as long as broad; frons shallow, marginate, with shallow central groove, 0.30x as broad as a head; head 0.33-0.35x as long as a rostrum, 2.20-2.30x as broad as a rostrum (Figs. 72, 117). Antennae scape short, not reaching to the middle of the eyes, 5.30-5.40x as long as broad, 0.93-1.00x as long as funicles; funicle segment I longer than other funicle segments 1.21-1.28x as long as segment II, 2.05-2.20x as long as segments III- V, segment VII 0.93-1.01x as long as segments I, funicle segment VII merged with the club; club 1.30-1.37x as long as broad, segment I 1.21-1.28x as long 0.83-0.85x as broad as segment II, segment 1.0-1.08x as long 1.38-1.42x as broad as segment III (Figs. 71, 145). Rostrum



Figs. 65-80. *Ornatalcides leopardus* Olivier, Habitus (65- 67) Male dorsal and lateral view; (68) Pronotum; Habitus (69- 70) Female dorsal and lateral view; (71) Antennae; (72) Male rostrum; (73) Female rostrum; Male genitalia (74- 75) Adeagus dorsal and lateral view; (76) Tegmen; (77) Male sternites VIII and IX, Female genitalia (78) Coxites and styli; (79) Spermatheca; (80) Female sternite VIII.

in dorsal view, 3.63-3.77x as long as broad, elongated cylindrically, almost parallel in width; scrobes almost parallel in width. (Figs. 117, 124).

Thorax: 0.79-0.85x as long as broad, shallow, ovate, constricted near to the apex, pronotum widest at base, 1.64-1.70x as broad as apex, surface of pronotum without any postocular lobes, covered with shallow small ovate punctations, ornamentations covered on both sides with creamy patches, with incomplete stripes at the middle (Figs. 68, 131). Scutellum small, slightly round shaped (Fig. 65). Elytra: shallow, ovate, widest at the shoulder, thereafter almost parallel in width from the base to near to the apex, covered with yellowish patches at equal interval of each interstriae, 1.73-1.80x as long as broad, 2.44-2.60x as long as rostrum, 2.39-2.45x as long as pronotum, convex at the apex (Figs. 65, 138). Legs: Foreleg longer than other legs, femur 3.58-3.70x as long as broad, femoral tooth 0.97-1.05x as long as broad, tibia 5.25-5.40x as long as broad with mucro, premucro well developed, claws well developed (Figs. 67, 152). Abdomen: with almost horizontally straight

ventrites, ventrite I shallow, dilated at the middle, 1.15-1.23x as long as broad, ventrite I 2.28-2.36x as long as II, 3.01-3.10x as long as III, 3.41-3.50x as long as IV, 1.98-2.08x as long as ventrite V, ventrite V shallow convex (Fig. 67).

Male genitalia: Aedeagus 7.31x as long as broad (Figs. 74, 75); aedeagal apodeme 1.16-1.20x as long as median lobe; in dorsal view: median lobe sub- cylindrical, parallel in width, abruptly narrowed at the apex; endophallus transparent, shorter than aedeagus (Figs. 159, 173). Tegmen 1.41-1.52x as long as broad, circular, manubrium small (Figs. 76, 180). Sternite VIII (Fig. 77) divided into two hemisternites, transversely oriented, lamellate shaped; each hemisternite horizontally acuminate, sternite VIII, IX connected with an almost transparent membrane; sternite IX with basal plate looks like a bow, shallow, curved at the middle; spiculum gastrale 4.20-4.32x as long as broad, slender, shallow sinuate (Fig. 166). Female genitalia: Spermatheca with distal arm 1.0-1.12x as long as a proximal arm, the angle between arms acute, both nodulus and ramus projecting out, V- shaped, ramus projecting out, swollen, almost parallel in width as a distal arm, cornu shallow, curved , with shallow narrowed at the apex (Figs. 78- 79). Sternite VIII with spiculum ventrale 3.0-3.12x as long as broad, Y- shaped, sternite VIII divided into two hemisternites, round, transversally acuminate, membranous, 2.18-2.25x as long as broad; coxites shallow, dilated from the styli, membranous; styli relatively small, swollen, shallow, pointed, apically inserted with long setae. (Fig. 80).

Measurements (mm)

Male: SL: 14.0- 14.20; SW: 4.70- 4.80; PL: 2.50-2.65; PW: 3.20- 3.30; EL: 8.10- 8.60; EW: 4.70- 4.80; RL: 2.50- 2.60; RW: 0.70- 0.75; HL: 1.0- 1.05; HW: 1.60- 1.70. Female: SL: 15.0- 15.35; SW: 4.90- 5.08; PL: 3.20- 3.28; PW: 3.42- 3.45; EL: 8.45- 8.65; EW: 4.90- 5.08; RL: 2.85- 3.05; RW: 0.79- 0.85; HL: 1.28-1.36; HW: 1.85- 1.89.

Remarks

Antennae funicle segment I 2.5-2.62x longer than funicular segments III- VI. Eyes flat, almost ovate shaped. Pronotum with ornamentations. Elytra almost parallel in width, ornamented with small circular shaped yellowish patches. Legs with a femoral tooth, tibiae with uncus, claws bidentate with a long, pointed tooth.

Host plants

Brassica napus and *Mangifera indica*.

Distribution

India: Karnataka, Tamil Nadu.

Material examined

2♂, 1♀; INDIA: Karnataka, Banglore, N12.947801°/; E77.587539°, 914m, 08.V.1953, Coll. M.A.N. Rao. 1♀; INDIA: Tamil Nadu, Tiruchirappalli, N10.4818°/; E78.4108°, 412m, 16.I.1922, Coll. B. C. Shantappa.

6. *Sternuchopsis* (Heller)

Sternuchopsis pectoralis Boheman in Schoenherr, 1836: 618 (NPC)

Redescription

Body length 11.6- 12.4 mm, slightly ovate shaped. Rostrum elongated cylindrically, 1.06-1.12x longer than pronotum; in dorsal view: irregular, shallow longitudinal margins visible, Antennae: scape short in length 0.73-0.78x as long as funicles, funicular segment I and VII longer than other funicular segments. Head 0.53-0.58x longer than rostrum. Eyes flat, ovate. Pronotum without ornamentation, surface covered with oval postocular lobes. Scutellum small, shallow, triangular shaped. Elytra without ornamentation, striate with shallow longitudinal pits. Abdomen almost U- shaped, covered with pale yellowish setae, with horizontally straight ventrites, ventrite I longer than the other ventrites, ventrite III- IV almost equal in length, ventrite V 2.66-2.72x longer than the ventrite II- IV, 2.0-2.10x as long as ventrite V having yellowish erect setae at the margin. Legs; foreleg longer than other legs, femur widest at the tooth, with a non- serrated tooth, tibia with premucro well developed. Male genitalia aedeagus curved shaped. Tegmen elongated, manubrium very small. Sternite VIII horizontally oriented. Female genitalia spermatheca angle between arms acute. Sternite VIII with spiculum ventrale Y- shaped, sternite VIII divided into two hemisternites.

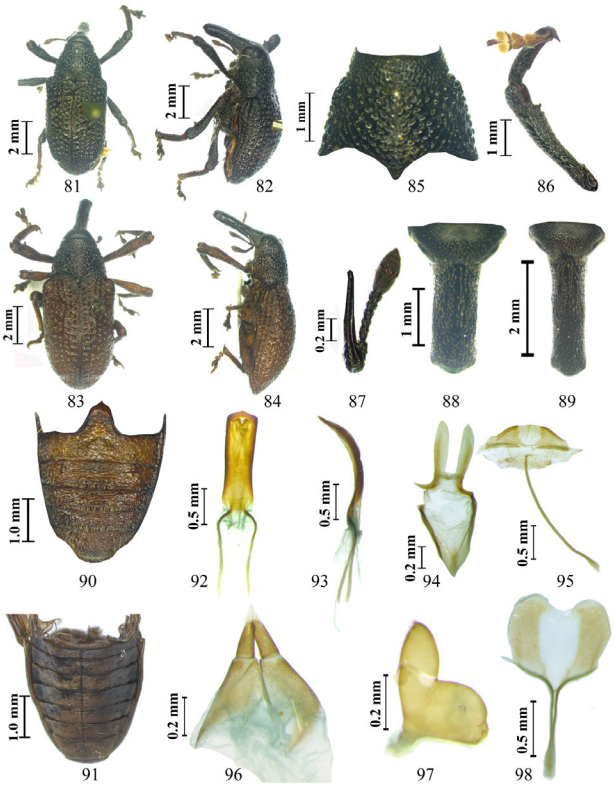
***Sternuchopsis pectoralis* (Boheman) (Figs. 81- 98)**

*Alcides pectoralis*Boheman in Schoenherr, 1836: 618; *Alcidodes pectoralis* Marshall, 1939: 570;

Sternuchopsis pectoralis Andrew and Ramamurthy, 2010: 309.

Redescription

Head: blackish with few recumbent yellowish-brown setae, frons covered with dense recumbent yellowish-brown setae; rostrum black in colour, irregularly marginate, at the base with few recumbent



Figs. 81-98. *Sternuchopsis pectoralis* Boheman, Habitus (81- 82) Male dorsal and lateral view; (83- 84) Female dorsal and lateral view; (85) Pronotum; (86) Foreleg; (87) Antennae; (88) Male rostrum; (89) Female rostrum; (90) Venter; (91) Sternum; Male genitalia (92- 93) Adeagus dorsal and lateral view; (94) Tegmen; (95) Male sternites VIII and IX; Female genitalia (96) coxites and styli; (97) Spermatheca; (98) Female sternite VIII.

yellowish-brown setae. Head round with shallow punctation, without markings, frons covered with sub-recumbent setae; 0.92-1.00x as long as broad; eyes flat, oval-shaped, 1.54-1.60x as long as broad, frons 0.32-0.38x as wide as head (Figs. 88, 118). Antennae scape reaching near to the middle of the eyes, 0.73-0.78x as long as funicle; segment I 1.14-1.20x as long as segment II and 1.60-1.70x as long as segment III–V, segment VII 1.14-1.20x as long and 1.60-1.65x as broad as segments III–V; club 1.45-1.52x as long as broad, segment I 2.0-2.12x as long and 1.22-1.25x as broad as segment II, segment II 0.5-0.55x as long and 1.50-1.52x as broad as segment III, in total club 2.67-2.78x as long and 1.0-1.05x as broad as segment I, 5.33-5.42x as long and 1.83-1.90x as broad as segment II, 2.67-2.72x as long and 1.83-1.90x as broad as segment III (Figs. 87, 146). Rostrum 4.25-4.40x, punctures at the apex, 1.25-1.28x as wide as frons, frons flat between eyes; in lateral view: scrobe starts from between apex and middle portion of the rostrum (Figs. 118, 125).

Thorax: 0.72-0.80x as long as wide, 1.64-1.70x as wide as apex, dorsal portion covered with ovate shaped postocular lobes, forming a shallow furrow vertically on the middle of pronotum (Figs. 85, 132). Elytra broadest at the shoulder, 1.33-1.40x as long as broad, 2.12-2.21x as long as a rostrum, 2.25-2.35x as long, 1.17-1.23x as broad as pronotum, apically convex; in lateral view moderately convex; in dorsal view U- shaped, elytra striate strongly converging towards the apex (Figs. 81, 139). Legs; Foreleg; femur 5.0-5.20x as long as broad, with a single tooth, tibia 6.33-6.38x as long as broad, with uncus, premucro well developed; in midleg, femur 4.35-4.52x as long as broad, with single mid femoral tooth; tibia 5.66-5.78x as long as broad, premucro well developed, corbels well developed compared to other two legs; in hindleg femur, 4.75-4.85x, tibia 5.86-6.02x as long as broad, corbels well developed, 3/4th of the leg bifurcate inside, claws connate (Figs. 86, 153). Abdomen: Surface of ventrite I shallow, depressed at the middle, ventrite II- IV almost horizontally straight, ventrite I 2.28-2.35x as long as II, 2.90-2.98x as long as III, 3.17-3.23x as long as IV, 2.14-2.20x as long as V, ventrite III and IV 1.09-1.15x subequal in length, ventrite V shallow, convex; hind coxae does not reach to the margin of elytra (Fig. 90).

Male genitalia: Aedeagus 5.27-5.40x as long as broad, in profile curved (Figs. 92- 93); aedeagal apodeme 0.86-0.92x as long as median lobe, apex of the aedeagus strongly sclerotised, membranous thereafter (Figs. 160, 174). Tegmen 4.20-4.28x as long as broad, elongated, manubrium very small (Figs. 94, 181). Sternite VIII (Fig. 95) horizontally oriented, shallow, arrow-shaped, pointed at middle. Sternite VIII, IX connected with almost transparent membrane; sternite IX with basal plate looks like a bow, shallow, curved at the middle, spiculum gastrale 4.65-4.72x as long as shaft, shallow curved at apex, thicker at the middle (Fig. 167). Female genitalia: Spermatheca with distal arm 0.89-0.95x as long as a proximal arm, angle between arms acute, nodulus circular shaped, ramus projecting out, cornu horizontally straight , with shallow, pointed at the apex (Fig. 97). Sternite VIII with spiculum ventrale 1.77-1.82x as long as broad, Y- shaped, sternite VIII divided into two hemisternites, elongated shaped, transversally acuminate, membranous with shaft elongate, 1.49-1.52x as long as basal plate, without setae (Figs. 98); coxites shallow dilated from the styli, membranous; styli relatively small, swollen, apically inserted with long setae (Fig. 96).

Measurements (mm)

Male SL: 11.60- 12.40; SW: 4.40- 4.70; PL: 3.00-

3.20; PW: 3.60- 3.85; EL: 6.50- 6.70; EW: 4.40- 4.70; RL: 2.20- 2.30; RW: 0.62- 0.65; HL: 1.25- 1.30; HW: 1.36- 1.40. Female: SL: 12.6.- 13.40; SW: 4.65- 4.88; PL: 3.10- 3.30; PW: 3.70- 3.85; EL: 6.70- 6.85; EW: 4.65- 4.88; RL: 2.50- 2.75; RW: 0.60- 0.64; HL: 1.28- 1.35; HW: 1.42- 1.48.

Remarks

Funicle segment VII continuous with the club. Pronotum without ornamentations, covered with oval shaped postocular lobes. Legs, femur with non- serrated tooth, premucro well developed, claw well developed.

Host plants

Ipomoea batatas.

Distribution

India: Tamil Nadu.

Material examined

3♂, 1♀, INDIA: Tamil Nadu, Coimbatore, N11.286208°/; E76.796644°, 749m, 18.III.1913, Coll. A.G.R.

7. *Tuberculomecyslobus* (Pajni and Dhir)

Tuberculomecyslobus Pajni and Dhir, 1987: 31.

Redescription

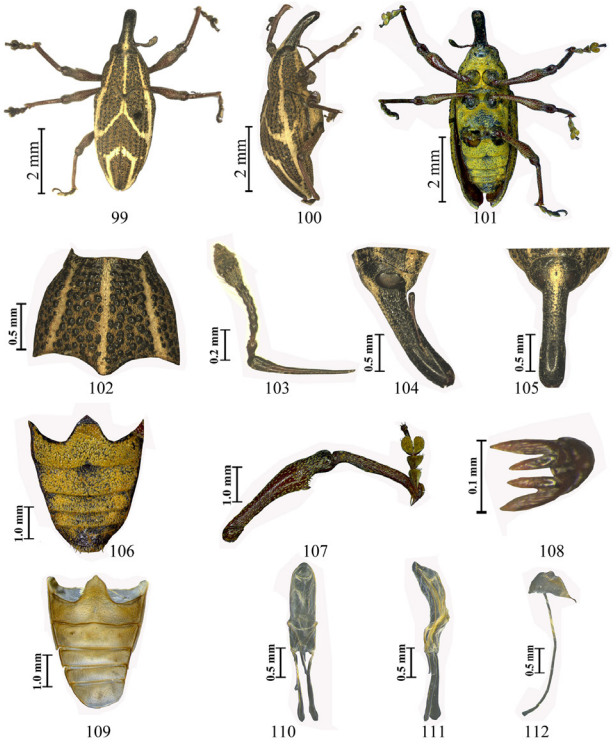
Body length 12.0- 12.60 mm, shallow ovate, pointed at elytral apex. Rostrum elongated cylindrically, frons covered with pale yellowish ornamentations, scrobes almost parallel in width at the middle of the rostrum. Eyes flat, ovate. Head with yellowish marking, without any punctations. Pronotum ornamented with 3 pale yellowish longitudinal stripes. Scutellum very small, triangular-shaped. Elytra in dorsal view: shallow ovate shaped, ornamented, with yellowish, wide striped marking which connects at the middle of both elytra, striate with black shiny postocular lobes. Abdomen shallow, U- shaped, with horizontally straight ventrites, covered with recumbent setae. Legs slender, foreleg longer than other legs, femur widest at the tooth, tibia with well- developed mucro, tarsi with well- developed claw. Male genitalia aedeagus curved shaped. Sternite VIII shallow, depressed at the middle, convex, pointed on both sides at the bottom, spiculum gastrale 3.67- 3.75x as long as broad sternite VIII.

***Tuberculomecyslobus crinalifer* (Marshall) (Figs. 99- 112)**

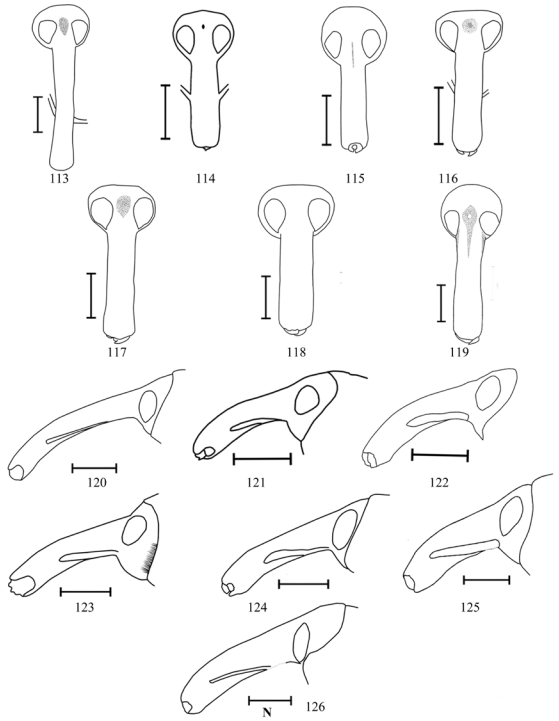
Alcidodes crinalifer Marshall, 1922: 393; 1939: 570; *Tuberculomecyslobus crinalifer* Pajni and Dhir, 1987: 31.

Redescription

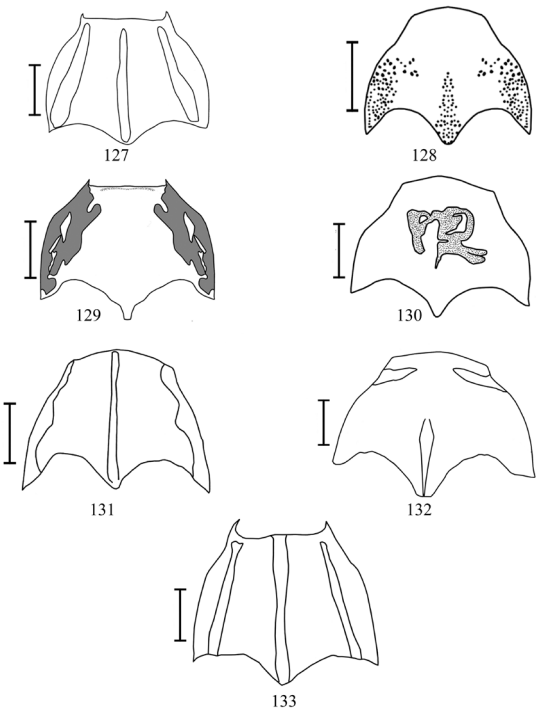
Head: Integument blackish, surface of frons covered with yellowish patch; antennae dark reddish brown, funicular segment VII covered with long brown erect setae; rostrum apex blackish; head moderately flat, 0.63- 1.68x as long as broad, frons covered with yellowish marking with a central longitudinal shallow groove; eyes flat, oval- shaped, frons 0.37-0.42x as wide as head (Figs. 105, 119). Antennae scape short, not reaching to the middle of eyes, scape 0.85-0.90x as long as funicles; segment I 1.17-1.25x as broad as segment II, segments III- V subequal in length, 0.58-0.62x as long as segment II, segment VII 2.17-2.30x as long and 2.00-2.14x as wide as segments III- V, segment VII 1.71-1.80x as wide as segments II- VI and 1.50-1.52x as wide as segment I, funicular segment VII longer than other funicular segments, covered with greyish erect setae; club 1.47- 1.52x as long as broad, segment I and II equal in length, segment II 0.78-0.84x as long as III (Figs. 103, 147). Rostrum 3.57-3.65x as long as wide, apical half portion



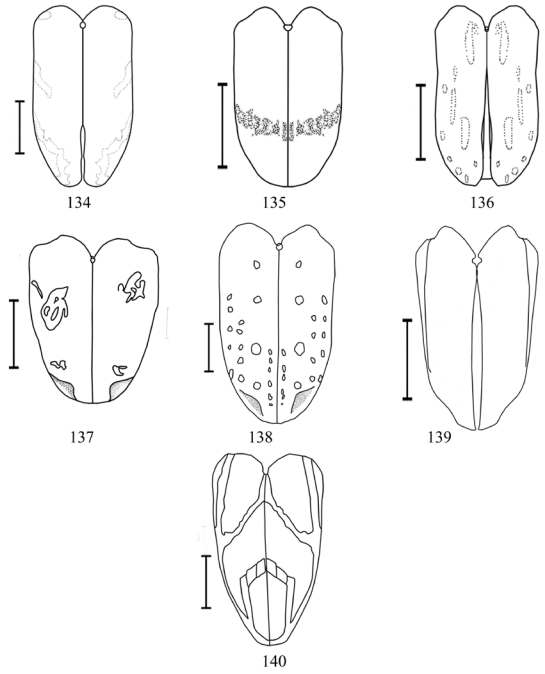
Figs. 99-112. *Tuberculomecyslobus crinalifer* Marshall, Habitus (99- 101) Male dorsal, lateral and ventral view; (102) Pronotum; (103) Antennae; (104- 105) Rostrum lateral and dorsal view; (106) Venter; (107) Forleg; (108) Claw; (109) Venter; Male genitalia (110- 111) Adeagus, dorsal view and lateral view; (112) Male sternites VIII and IX.



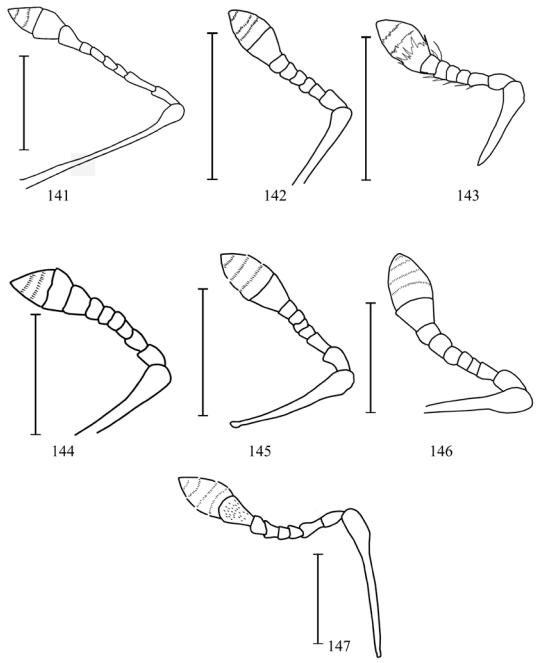
Figs. 113-126. Head, Rostrum dorsal and lateral view (113, 120) *Merus fasciatus* Gistel; (114, 121) *Brahamalcidodes micronychus* Pascoe; (115, 122) *Cylindralcides bubo* Fabricius; (116- 123) *Neomecyslobus feae* Faust; (117, 124) *Ornatalcides leopardus* Olivier; (118, 125) *Sternuchopsis pectoralis* Boheman; (119, 126) *Tuberculomecyslobus crinalifer* Marshall. Scales: 1.0 mm.



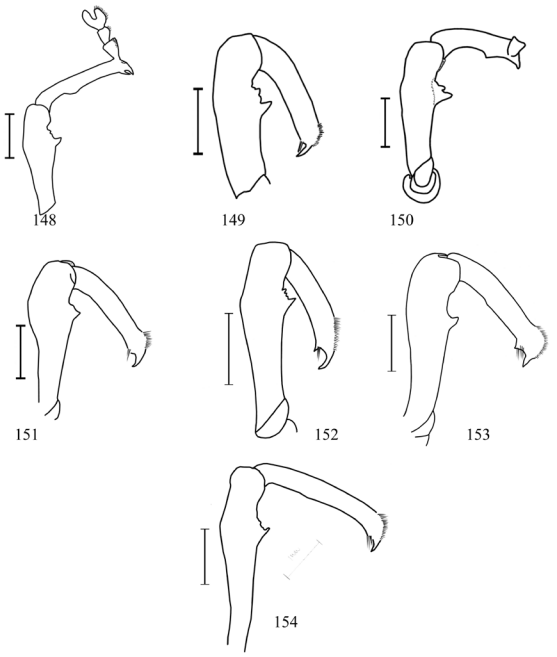
Figs. 127-133. Pronotum (127) *Merus fasciatus* Gistel; (128) *Brahamalcidodes micronychus* Pascoe; (129) *Cylindralcides bubo* Fabricius; (130) *Neomecyslobus feae* Faust; (131) *Ornatalcides leopardus* Olivier; (132) *Sternuchopsis pectoralis* Boheman; (133) *Tuberculomecyslobus crinalifer* Marshall. Scales: 1.0 mm.



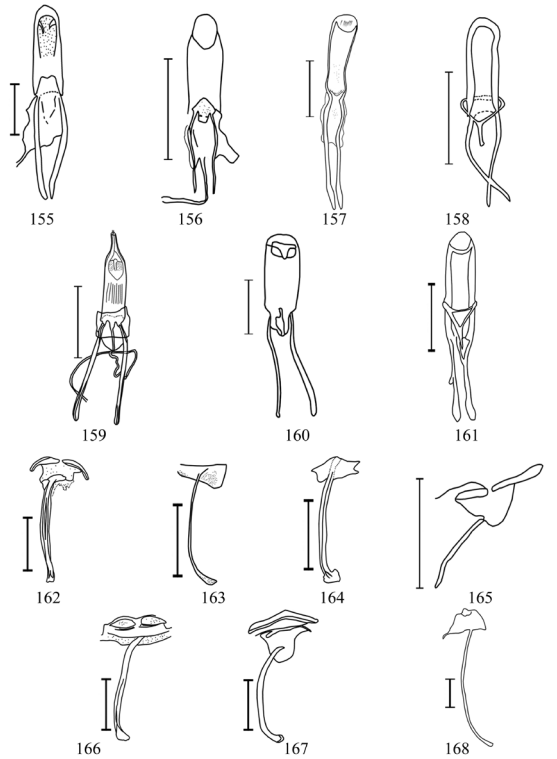
Figs. 134-140. Elytra (134) *Merus fasciatus* Gistel; (135) *Brahamalcidodes micronychus* Pascoe; (136) *Cylindralcides bubo* Fabricius; (137) *Neomecyslobus feae* Faust; (138) *Ornatalcides leopardus* Olivier; (139) *Sternuchopsis pectoralis* Boheman; (140) *Tuberculomecyslobus crinalifer* Marshall. Scales: 2.0 mm.



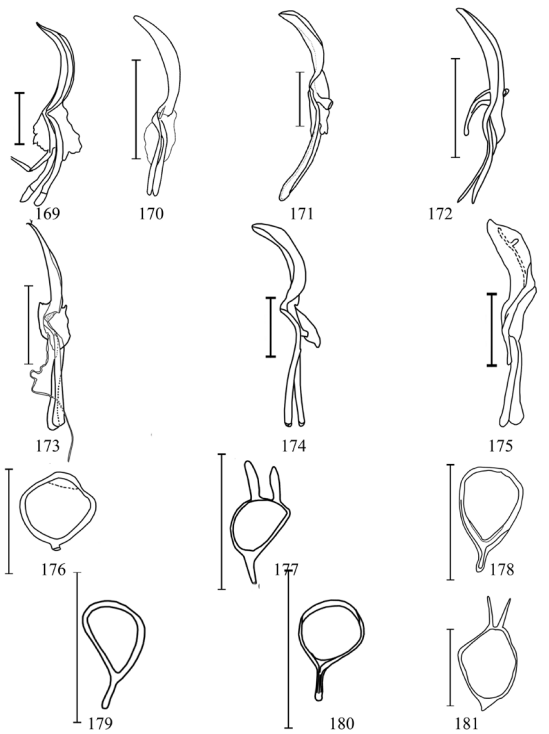
Figs. 141-147. Antennae (141) *Merus fasciatus* Gistel; (142) *Brahamalcidodes micronychus* Pascoe; (143) *Cylindralcides bubo* Fabricius; (144) *Neomecyslobus feae* Faust; (145) *Ornatalcides leopardus* Olivier; (146) *Sternuchopsis pectoralis* Boheman; (147) *Tuberculomecyslobus crinalifer* Marshall. Scales: 1.0 mm.



Figs. 148-154 Foreleg (148) *Merus fasciatus* Gistel; (149) *Brahamalcidodes micronychus* Pascoe; (150) *Cylindralcides bubo* Fabricius; (151) *Neomecyslobus feae* Faust; (152) *Ornatalcides leopardus* Olivier; (153) *Sternuchopsis pectoralis* Boheman; (154) *Tuberculomecyslobus crinalifer* Marshall. Scales: 1.0 mm.



Figs. 155-168. Male genitalia, aedeagus and sternite (155, 162) *Merus fasciatus* Gistel; (156, 163) *Brahamalcidodes micronychus* Pascoe; (157, 164) *Cylindralcides bubo* Fabricius; (158, 165) *Neomecyslobus feae* Faust; (159, 166) *Ornatalcides leopardus* Olivier; (160, 167) *Sternuchopsis pectoralis* Boheman; (161, 168) *Tuberculomecyslobus crinalifer* Marshall. Scales: 1.0 mm.



Figs. 169-181. Male genitalia, aedeagus and tegmen (169, 176) *Merus fasciatus* Gistel; (170, 177) *Brahamalcidodes micronychus* Pascoe; (171, 178) *Cylindralcides bubo* Fabricius; (172, 179) *Neomecyslobus feae* Faust; (173, 180) *Ornatalcides leopardus* Olivier; (174, 181) *Sternuchopsis pectoralis* Boheman; (175) *Tuberculomecyslobus crinalifer* Marshall. Scales: 1.0 mm.

of the rostrum with a smooth surface, widest at the apex, 1.35x as wide as frons (Figs. 104- 105).

Thorax: Transverse, 0.79-0.83x as long as wide, broadest at the base, with the sides gently rounded, shallowly constricted at the apex, 1.65-1.72x as broad as apex, covered with recumbent greyish setae, with ornamentation having longitudinal stripes on both sides, at the middle with yellowish- grey setae, covered with postocular lobes, black in colour, postocular lobes well developed (Figs. 102, 133). Scutellum heart- shaped, black (Fig. 99). Elytra elongated, less ovate, 3.34-3.40x as long as broad, 2.77-2.85x as long as rostrum, 2.83-2.92x as long, 1.43-1.52x as broad as pronotum, elytra having ornamentation with a yellowish stripe starting just before the shoulder from the base of the prothorax, continued concavely towards the apex of elytra; another yellowish stripe starting from scutellum, runs diagonally, meet the first one in midway, the third stripe is a patch of hairs starting from interval 1, 2 and 3 at a distance of 2/3rd from the elytral base, the 3rd stripe from interval 3 runs vertically towards the base of elytra, join the 1st interval just before the base (Figs. 99, 140). Foreleg femora slender, with a serrated tooth with 2- 3

teeth, femur width more at the tooth, 3.23-3.30x as long as broad with the apices of the femora, tarsi blackish; tibia with mucro well- developed, premucro absent with open type corbels, all the femora with a sharp tooth, with a row of serrated teeth (Figs. 107, 154). Abdomen: Integument blackish in colour, covered with yellowish dense recumbent setae 1.32-1.42x as long as broad, ventrite I shallow, depressed at the middle; ventrite I, 1.55-1.62x as long as II; ventrite II, 1.50-1.58x as long as ventrite III; 2.0-2.15x as long as ventrite IV; 1.38-1.42x as long as ventrite V; ventrite V broadly parabolic, convex with pointed spike- like erect setae, procoxae far from margin of prosternum, 0.41-0.45x as wide as a metacoxae; the metacoxae apart by 0.44-0.48x as wide as hind coxae, hind coxae almost reaching margin of elytra (Figs. 106, 109).

Male genitalia: Aedeagus 6.0-6.25x as long as broad, curved (Figs. 110- 111); aedeagal apodeme 0.80-0.85x as long as median lobe, median lobe cylindrical, almost parallel in width from apex to the base, apex of the aedeagus membranous, and shallow, pointed (Figs. 161, 175). Sternite VIII (Fig. 112) shallow, depressed at the middle, convex, pointed on both sides at the bottom, spiculum gastrale 3.67-3.75x as long as broad 7.33-7.40x as long as sternite VIII (Fig. 168).

Measurements (mm)

Male: SL: 12.0; SW: 3.64; PL: 2.76; PW: 3.48; EL: 6.96; EW: 4.16; RL: 3.40; RW: 0.95; HL: 1.20; HW: 1.90.

Remarks

Antennae, funicle segment VII 1.08-1.12x longer than funicle segments I- II and separate from the club. Pronotum with ornamentations, with longitudinal stripes, covered with black postocular lobes. Elytra ornamented with stripes, covered with black postocular lobes.

Distribution

India: Tamil Nadu.

Material examined

1♂, INDIA: Tamil Nadu, Niligiri hills, Kallar, N11.3744°/; E76.7620°, 2014m, 14.IX.1952, Coll. P. Susai Nathan.

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BEHAVIOURAL RESPONSE OF THE PARASITOID *COTESIA FLAVIPES* TO HERBIVORE INDUCED VOLATILES IN SWEET SORGHUM

S Shwetha and P G Padmaja*

ICAR- Indian Institute of Millets Research, Rajendranagar, Hyderabad 500030, Telangana State, India
*Email: padmaja@millets.res.in (corresponding author)

ABSTRACT

Plants emit a variety of compounds in response to an attack by herbivores. Herbivore-induced plant volatiles (HIPVs) mediate interactions between plants and natural enemies. Volatiles were collected from sweet sorghum plants oviposited by *Chilo partellus* Swinhoe and the response of the parasitoid *Cotesia flavipes* Cameron to these volatiles were tested in four-arm olfactometer. *Cotesia flavipes* spent significantly more time (6.52± 0.72 min, p = 0.0000) in arm treated with *C. partellus* oviposited plant volatiles compared to untreated control (3.17± 0.19 min). These compounds were identified by GC-MS as octanal, decanal, nonanal, 6-methyl 5 heptanone and caryophyllene. Nonanal and decanal were 8.46 and 4.66%, respectively in plants with *Chilo* eggs, whereas in the control plants, it was 5.48 and 1.39%, respectively. The behavior of parasitoid towards HIPVs enhances the development of sustainable IPM strategies by manipulating the foraging behaviour of parasitoids.

Key words: Sweet sorghum, spotted stemborer, oviposition, air entrainment, volatiles, nonanal, caryophyllene, decanal, parasitoid, attraction, olfactometer, behavioural assay

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is an annual, C4 crop with sugar-rich stalks and characterized by a high photosynthetic efficiency (Lingle et al., 2012; Murray et al., 2009; Smith et al., 1987). It provides both grain and stem which will be used for sugar, alcohol, syrup, jaggery, fodder, fuel, etc.; and there are about 4,000 sweet sorghum cultivars (Rutto et al., 2013). Crop productivity is severely affected due to stem borer species observed as serious pests in Asia and Africa, of which the spotted stem borer (*Chilo partellus* Swinhoe) is the most destructive. It occurs throughout the crop growth and development, both in Asia and Africa. Within the semiarid tropics alone it causes US$ 334 million annual loss to sorghum (Sharma, 2006). The major components of IPM are cultural practices, insecticides, biological control and host plant resistance. Chemical control is expensive and often beyond the reach of resource poor farmers. Insecticides are also ineffective for stem borer control because larvae bore into the shoot and pupate within which makes them hard to target (Khan et al., 2000). Under such circumstances, host plant resistance is the best method, and considerable progress has been made in developing techniques to screen for resistance and in identifying mechanisms of resistance. However, to date stem borer resistance has not yet been bred into high yielding cultivars. Stem borer larvae have a broad range of natural enemies which are able to locate and attack the larvae that feed inside the plant tissue. These biological control agents are successful based on their efficiency to search and locate target hosts (Nordlund et al., 1988).

Plants have evolved sophisticated defense mechanisms which protect against insect attack. They respond to insect attack by releasing a blend of volatiles that serve as foraging cues for parasitoids. Parasitoids use volatile compounds released by insect herbivore-damaged plants to locate their hosts. These volatiles can be exploited to attract parasitoids to improve biological control in the field. Volatile chemical compounds from the host plant and the herbivores, or the interaction of the two, play an important role (Dicke, 1994), and to locate their hosts during foraging parasitoids utilize volatiles cues (Finidori-Logli et al., 1996; Potting et al., 1995; Steinberg et al., 1993; Vet and Dicke, 1992). The host plant volatile profile thus plays a key role in attracting or repelling or retaining the natural enemies (Vinson, 1975; Gohole et al., 2003). In tritrophic systems consisting of plants, herbivorous arthropods and their carnivorous natural enemies, carnivores are attracted to volatile compounds emitted by plants infested by herbivores (Takabayashi and Dicke, 1996; Arimura et al., 2009). Plant odours are used as cues by the parasitoids and predators (Vinson, 1976, 1981; Nordlund et al., 1988). These odours are inducible and only released after damage by herbivores and are termed as herbivore induced plant volatiles (HIPVs).

The quantity and the composition blend of compounds emitted by plants vary with the herbivore, the plant species and the genotype. The compositions of these volatiles are specific, depending on the species and cultivars (Takabayashi and Dicke, 1996; Arimura et al., 2009). These herbivore species-specific HIPVs facilitate the location of host-infested plants by parasitoids (Vet and Dicke, 1992); and their production is triggered on feeding damage by herbivorous larvae. Plant responses to an earlier stage of insect attack (egg deposition) (Colazza et al., 2004; Hilker and Meiners, 2006; Bruce et al., 2009; Tamiru et al., 2011). Defenses elicited due to eggs benefit plants as they enable defense to be switched on early, before leaf or stem damage is caused by larvae (Hilker and Meiners, 2006; Bruce et al., 2009). The tritrophic interactions presents an opportunity for development of cost effective and environmentally benign IPM approaches. Herein, role of chemical cues emanating from the sweet sorghum plants oviposited by *C. partellus* in the acceptance of host by searching parasitoid *C. flavipes* has been explored.

MATERIALS AND METHODS

Sweet sorghum plants (cv. 'Wray') were grown individually in pots in a nethouse. The plants were grown in plastic pots (13x 14 cm dia). Plants at the stage of five fully grown leaves were used for the volatiles collection. Plants were introduced into the cages with six *C. partellus* females and removed 24 hr later. On an average, 12 eggs per 5 plants were laid. Sweet sorghum plants with eggs were used for the collection of volatiles. Intact plants were used as controls. The *C. partellus* was maintained in the laboratory on semisynthetic diet under controlled conditions (27°C, 70% RH, 12:12 light-dark photoperiod) as described by Padmaja et al., 2012. The parasitic wasp, *C. flavipes* was reared on larvae of *C. partellus*, with the appropriate stages removed from artificial diet and fed for 24 hr on pieces of sorghum stem for acclimatization; larvae were exposed to 24 hr old mated *Cotesia* females for oviposition using the hand-stinging method; only one stinging was allowed/ larva and adult parasitoid. Parasitized larvae were placed individually in a glass vial containing a sorghum stem piece, and plugged with cotton; and these vials incubated at 27°C, at 70% RH, and 12L: 12D photoperiod for the parasite emergence. Volatiles from the sweet sorghum cultivar "Wray" grown individually in pots in a nethouse were entrained. Multiple collections were made with portable equipment that allows sampling of volatiles, for 21 days after seedling emergence, the most susceptible stage (Padmaja et al., 2010). Aliquots of attractive headspace samples were analyzed on a Gas Chromatography (GC) - Agilent technologies 7890A system equipped with 7000 GC-MS Tripple quad with column (DB - 5 MS, 30 m length, 0.25 mm i.d., 0.50 µm film thickness) directly coupled to a mass spectrometer. The oven temperature was maintained at 30°C for 5 min, rate 5°C/min to 250°C hold for 11 min. Compounds were identified by comparison of retention indices and mass spectra with those of authentic standards 6-methyl 5 heptanone, octanal, decanal, nonanal and caryophyllene obtained from Sigma Aldrich. Responses of parasitoids to plant derived volatiles were tested in a perspex four-arm olfactometer (Padmaja et al., 2010). Air was drawn through the four arms towards the center at 260 ml min^{-1}. Headspace samples (10 µl) were applied to a piece of filter paper with a micropipette and placed in an inlet port at the end of each olfactometer arm; and freshly emerged *C. flavipes* were transferred individually into the central chamber, and time spent in different regions was compared. A choice test to compare insect responses to headspace samples from oviposition induced and control (unexposed) plants was carried out by placing the test stimuli (10 µl aliquots of headspace sample) in two opposite arms. The other two arms contained filter paper with 10 µl diethyl ether, and were used as controls. Time spent in each region was recorded. Ten replicates were carried out. A paired-sample t-test was employed to analyze the differences between the time spent by *C. flavipes* in each arm of the olfactometer.

RESULTS AND DISCUSSION

Coupled gas chromatography-mass spectrometry revealed that the sweet sorghum genotype 'Wray' emitted more volatile compounds when exposed to *C. partellus* eggs compared to unexposed plants (Fig. 1). Major components that have been identified in both plant categories were 6-methyl 5 heptanone, octanal, decanal, nonanal and caryophyllene. Nonanal being the most abundant volatile compound emitted both in intact and in *C. partellus* oviposited plants. Significant differences in the abundance of nonanal, decanal, caryophyllene and octanal were observed in the headspace profile between intact plants and plants with *C. partellus* eggs. More specifically, nonanal and decanal were 8.46 and 4.66%, respectively, in plants with *Chilo* eggs, whereas in the control plants, it was 5.48 and 1.39%, respectively (Table 1). Female *C. flavipes*, spent significantly more time in the region with volatiles from 'Wray' exposed to oviposition by

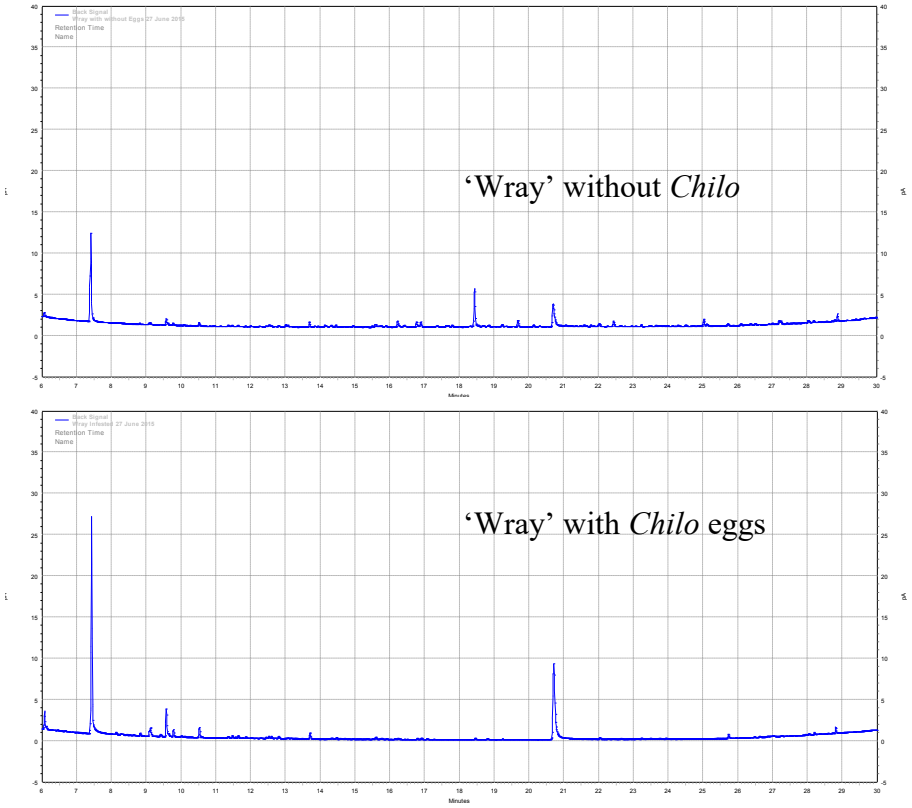

Fig. 1. Gas chromatogram (GC) traces of compounds in headspace samples of sweet sorghum

Table 1. Identification of volatile compounds (GC-MS) in sweet sorghum cultivar 'Wray'

Peak	Without *Chilo* eggs			Peak	With *Chilo* eggs		
	RT	Area %	ID of compound		RT	Area %	ID of compound
1	16.938	2.08	6-methyl 5 heptanone	1	16.928	3.61	6-methyl 5 heptanone
2	17.614	1.16	Octanal	2	17.604	3.33	Octanal
3	21.014	5.48	Nonanal	3	21.004	8.46	Nonanal
4	24.201	1.39	Decanal	4	24.153	4.66	Decanal
5	30.102	2.28	Caryophyllene	5	30.092	2.84	Caryophyllene

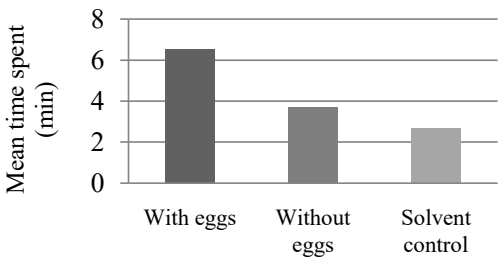

Fig. 2. Behavioural response of *C. flavipes* in a four-arm olfactometer bioassay to volatiles collected from sweet sorghum (Wray) plants (n=10)

C. partellus, compared to regions with unexposed and blank controls ($p<0.0000$; $df = 9$) (Fig. 2). The increase in time spent is a positive response indicating that attraction and arrestment of parasitoids increased. A number of studies have shown that OIPVs serve as cues for foraging parasitoids (Hilker and Fatouros, 2015; Colazza et al., 2004; Tamiru et al., 2012). Although it was first considered as a plant's response to wound oviposition (Hilker and Fatouros, 2015), later studies have shown that oviposition itself is responsible for the induction of qualitative and quantitative changes in the volatile profile of egg infested plants (Tamiru et al., 2011; Anastasaki et al., 2015). Plants benefit by an early activation of defense mechanisms by egg deposition, which enhances their defense before any damage can occur (Hilker and Fatouros, 2015; Bruce et al., 2009). The behavioral response of egg parasitoid *Trichogramma achaeae* females to HIPVs produced by tomato plants

infested with *Tuta absoluta* eggs or larvae in tomato when studied revealed that oviposition and larval feeding by *T. absoluta* significantly enhanced HIPV emission. The analysis of volatiles released by tomato plants, either infested or uninfested, coupled with the response of *T. achaeae* in the olfactometer tests was consistent with what was expected in terms of the foraging behavior of a generalist parasitoid (Gontijo et al., 2019).

The present study demonstrated quantitative variations in volatiles in sweet sorghum cultivar 'Wray' when *C. partellus* eggs were laid; and a preference was observed in an olfactometer bioassay of *C. flavipes* parasitoids for volatiles from plants exposed to egg deposition (Fig. 2). Attraction of larval parasitoids means that natural enemies can also attack newly hatching larvae. Volatiles emitted from apple leaves infested by two-spotted spider mite *Tetranychus urticae* attracted the *P. persimilis* and *Metaseiulus occidentalis* (Sabelis and Van de Baan, 1983). Upon infestation by *T. urticae*, Lima bean plants emitted a blend of volatiles attracting the predatory mite *P. persimilis* (Dicke et al., 1990 a,b). Corn plants damaged by *Spodoptera exigua* emitted volatiles that attracted the parasitoid *Cotesia marginiventris* Cresson (Turlings et al., 1990). Several behavioral and electrophysiological studies had revealed the attractiveness of HIPVs to predators (Drukker et al., 1995; Zhang et al., 2009; Zhang et al., 2012) and parasitoids (Turlings and Tumlinson, 1992; Yu et al., 2008; Yu et al., 2010). Maize plants under attack by larvae of *S. littoralis* attracted *C. marginiventris* and *Campoletis sonorensis* Cameron which resulted in higher parasitization and reduced feeding and weight gain of the host larvae (Hoballah and Turlings, 2001). Parasitized larvae attacked plants produced 30% more seeds than plants attacked by unparasitized larvae. Insect parasitoids are known to utilize the different volatile profile of infested plants vs non-infested plants to detect their hosts and prey. The present study is one of the first in which the egg induced volatile emission effect is studied in sweet sorghum. It is proved that the parasitoid *C. flavipes* responds to oviposition-induced volatiles released by the plants after oviposition by a herbivore. HIPVs provide parasitoids with early alert cues to enhance their foraging efficacy.

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AVIFAUNAL DIVERSITY IN MUSTARD CROP

Amanpreet Kaur and Manoj Kumar*

Department of Zoology, Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: mansnr@pau.edu (corresponding author)

ABSTRACT

Avifaunal diversity in mustard (*Brassica spp*.) crop was studied from October 2020 to April 2021 in the agricultural field areas at two locations of district Ludhiana, Punjab. A total of 40 species were observed, with the most dominant order and family being Passeriformes (57%) and Passeridae (17.5%), respectively. Based on the status of birds, 75% were resident, 17.5 % were resident migrant and 7.5% were migrant. As per IUCN status, all 40 species falls under least concern category. As the foraging guilds of the birds are overlapping resulting in some birds falling under two or more foraging guilds and as per their foraging habits, 52% were insectivorous, 14% were frugivorous, 11% granivorous, 9.5% feeding on small invertebrates, 8.5% phytophagous, 4% feeding on small vertebrates and 1% omnivorous. Based on the status of birds, 75% birds were resident, 17.5 % were resident migrant and 7.5% were migrant.

Key words: Bird diversity, oil seed crop, phonological stages, insectivores, foraging guild, resident status, least concern, relative abundance, richness, evenness

Punjab is a state in northwest region covering 1.5% topographical space of India and at the same time gives food to 13-14% of population of India (McLaughlin 2011). Oilseed crops, accounted around 19% of worldwide area together with 2.7% of worldwide yield, hold the second most significant determining factor of Indian rural economy sector close to whole grains (Madhusudhan, 2015, Reddy and Emmanuel, 2017). Mustard is an essential Rabi season oil-rich seed plant, being a part of family Brassicaceae and belongs to genus *Brassica* and it need less water for finishing lifecycle, thus it is the most sustainable cropping system under rain fed cropping system (Anonymous, 2021). In an agroecosystem, birds serve a dual purpose therefore; they are useful and harmful. Birds are of huge worth to mankind due to their annihilation of bugs and seed of weeds, assume a critical part in the seed dispersal and flower pollination (Pitti et al., 2016; Mangan et al., 2017). Intensive agricultural practices and reduction in natural habitats leads to dependence of birds on agricultural crops for food and the significance of birds in agribusiness has been studied by Ali (1978). Birds are endothermic (warm-blooded), bipedal, egg-laying, padded, winged and vertebrate creatures. They have an incredible variety of conduct, shading, structure and size. There are 1306 species of birds reported from India, among them 72 (5.5%) are endemic, 26 are uncommon or incidental. Systematically, it cover-up 26 orders, 111 families and 492 genera (Pitti et al., 2016). The total avifauna in Punjab involves 328 types of bird (Jerath and Chadha, 2006). In addition to beneficial aspects of birds there are harmful aspects are also there so it is fundamental for discovering economical and regularly accessible technique to decrease bird depredation in crops (Firake et al., 2016). The present work was undertaken to study the avifaunal diversity in mustard crop.

MATERIALS AND METHODS

The present study was conducted in the selected mustard crop fields at two locations during Rabi season from October 2020 to April 2021. Location I (LI): Agricultural fields at Punjab Agricultural University campus (PAU), Ludhiana (75.44° E, 30.59° N, 229m above mean sea level. Location II (LII): University Seed Farm (USF), Ladhowal, Ludhiana (75. 49° E, 30.57° N, 189m above mean sea level). The crop was raised all according to cultural practices provided in Package and Practices for *Rabi* crops, Directorate of Extension, Punjab Agricultural University, Ludhiana (Anonymous 2021). Mustard seeds were sown by broadcasting method in standing water. No application of pesticide was done at any stage of the crop. Fields with half acre of area were selected in triplicates at both the locations at a distance of 500 m from each other at both locations. Prominent crops and plantations surrounding the selected fields at LI were wheat (*Triticum aestivum*), sunflower (*Helainthus annus*), guava (*Psidium guajava*), kinnow (*Citrus sinesnsis*) and poplar (*Populous deltoids*) and at LII were wheat (*Triticum aestivum*), Bajra *(Pennisetum glaucum),*

potato *(Solanum tuberosum)* and sagwan *(Tectona grandis)*. Observations were taken in the morning and evening depending upon the season, as birds has maximum activity during that time, on weekly basis. Species in the fields, on vegetations, on the ground and also utilizing perches like poles, electricity wires and trees around selected fields were recorded and identified based on visual observations which include their morphological characters such as color, size, wings, beak, shape and rest of body parts with the help of binocular (Nikon 10/50) and comparing with those described by Ali (2003). Standard nomenclature of birds was followed as per Manakadan and Pittie (2001). Birds were also categorized based on the foraging habits (Ali, 2003). The entire data of respective stages were pooled and community characteristics i.e. relative abundance, Shannon-Weiner Index of species diversity and species evenness were calculated (Krebs et al., 1985; Jerath and Chadha, 2006).

RESULTS AND DISCUSSION

The present study was conducted on mustard crop to study the avian diversity in different growth stages of crop and to access the damage and evaluation of bird pest management methods. A total of 40 species of birds were observed during the study on the selected locations. Nearly, most of the birds belongs to order Passeriformes. Also, two members each from order Pelecaniformes, Ciconiiformes Coraciiformes, Columbiformes and Cuculiformes. Three members belongs to order Galliformes. Also, only, one member each from order Charadriiformes, Bucerotiformes and Psittaciformes. Majority of the birds are resident (75%) but 17.5 % were resident migrant and 7.5% were migrant. As per IUCN status, all forty bird species fall under least concern category (Kler and Kumar, 2015). The most dominant order and family was Passeriformes (57%) and Passeridae (17.5%) respectively. Based on the status of birds, 76% of the bird spices observed were resident and 24% were resident migrant (Table 1). Birds were also categorized based on the foraging guilds (Ali, 2003). As the foraging guilds of the birds are overlapping which results in some birds were falling under two or more foraging guilds and as per their foraging habits, 52% were insectivorous, 14% were frugivorous, 11% granivorous, 9.5% feeding on small invertebrates, 8.5% phytophagous, 4% feeding on small vertebrates and 1% omnivorous (Table 1). Birds do have various dietary habits but for integrated pest management insectivorous birds are of farmer's interest. Dominant category of bird species present in Punjab are insectivorous so they are dependent on insects as prey (Losey and Vaughan, 2006; Kler and Kumar, 2015).

Avian diversity in relation to phenological stages of the mustard crop revealed that maximum bird species belongs to order Passeriformes (22), three members were from order Galliformes, followed by two species were from each order like Pelecaniformes, Ciconiiformes, Coraciiformes, Columbiformes and Cuculiformes. Only one member was from each family such as Charadriiformes, Bucerotiformes and Psittaformes (Table 2). As per their foraging guild, 35 insectivorous, 8 each under frugivorous, feeding on small invertebrates, 7 granivorous and 6 phytophagous and 1 omnivorous in feeding habits. Species richness was high in seedling stage and low during sowing stage where as species diversity was high in seedling stage while low in ripening stage. Kale et al. (2014) reported that in horticulture crop of pointed gourd Cattle Egret as well as Common Myna were seen predating the pest *Margaronia indica* and controlling the pest problem proving the benefits of insectivorous birds. Similarly at location II, 26 insectivorous, 7 frugivorous, 6 feeding small invertebrates, 5 granivorous, 4 phytophagous, 2 feeding on small vertebrates and 1 omnivorous bird species recorded. Maximum bird species belongs to order Passeriformes, followed by Muscicapidae. Species richness was maximum in vegetative and ripening stage while low in sowing and flowering stage. Species diversity was high in vegetative stage and low in sowing stage (Table 2).

The variable species richness and species diversity at different phenological stages of the crop at both locations was because of the presence of different types of vegetations as well as crops in the surrounding areas, as at location I there were different crops were present in surrounding fields whereas more uniform cropping pattern was observed at location II. Higher number of insectivorous birds were observed at both the locations (Table 2). Insectivorous birds were observed to be utilizing natural and artificial structures for perching purposes near the crop fields. They gleaned the fields from these perching places before foraging the crop. Other workers also observed such behavior that birds in crop fields which were supported by perches and insectivorous birds are height generalist which relies on structure of vegetation, abundance and distribution of prey; also, it affects their perch height selection (Narayana et al., 2014; Ali et al., 2010). Karp et al. (2013) revealed that a few avifaunal species can annihilate psyllids from woodland areas. Martin

Table 1. Avian diversity in mustard crop- Ludhiana (rabi, October 2020-April 2021)

No.	Common name	Scientific name	Order	Family	Status	Feeding habits
1.	Asian pied starling	*Sturnus contra*	Passeriformes	Sturnidae	R	I, F
2.	Bank myna	*Acridotheres ginginianus*	Passeriformes	Sturnidae	R	I, F
3.	Black drongo	*Dicrurus macrocercus*	Passeriformes	Dicruridae	R	I
4.	Black francolin	*Francolinus francolinus*	Galliformes	Phasianidae	R	G, I
5.	Black ibis	*Pseudibis papillosa*	Pelecaniformes	Threskiornitiidae	R	I, G
6.	Black kite	*Milvus migrans*	Ciconiiformes	Accipitridae	R	I, R
7.	Black redstart	*Phoenicurus ochruros*	Passeriformes	Muscicapidae	RM	I
8.	Black-winged Stilt	*Himantopus himantopus*	Charadriiformes	Recurvirostridae	R	I
9.	Blue rock pigeon	*Columbia livia*	Columbiformes	Columbidae	R	G
10.	Brainfever bird	*Hierococcyx varius*	Cuculiformes	Cuculidae	R	I, F
11.	Cattle egret	*Bubulcus ibis*	Pelecaniformes	Ardeidae	RM	I, SI
12.	Common babbler	*Turdoides caudatus*	Passeriformes	Silvidae	R	I, F
13.	Common hoopoe	*Upupa epops*	Bucerotiformes	Upupidae	RM	I
14.	Common myna	*Acridotheres tristis*	Passeriformes	Sturnidae	R	I, F
15.	Common starling	*Sturnus vulgaris*	Passeriformes	Sturnidae	M	I, F
16.	Common tailor bird	*Orthotomus sutorius*	Passeriformes	Cisticolidae	R	I, P
17.	Eurasian collared dove	*Streptopelia decaocto*	Columbiformes	Columbidae	R	G
18.	Greater coucal	*Centropus sinensis*	Cuculiformes	Centropodidae	RM	I, SI, SV
19.	Greenish leaf -warbler	*Phylloscopus trochiloides*	Passeriformes	Silvidae	M	I
20.	Grey francolin	*Francolinus pondicerianus*	Galliformes	Phasianidae	R	G, I
21.	Grey wagtail	*Motacilla cinerea*	Passeriformes	Passeridae	M	I, F
22.	House crow	*Corvus splendens*	Passeriformes	Corvidae	R	O
23.	House sparrow	*Passer domesticus*	Passeriformes	Passeridae	R	G, I
24.	Indian pea fowl	*Pavo cristatus*	Galliformes	Phasianidae	R	G, P, I, SV
25.	Indian robin	*Saxicoloides fulicata*	Passeriformes	Muscicapidae	R	I
26.	Indian roller	*Coracias benghalensis*	Coraciiformes	Coraciidae	R	I
27.	Jungle babbler	*Turdoides striatus*	Passeriformes	Silvidae	R	I, F
28.	Large pied wagtail	*Motacilla maderaspatensis*	Passeriformes	Passeridae	R	I, SI
29.	Oriental magpie robin	*Copsychus saularias*	Passeriformes	Muscicapidae	R	I
30.	Oriental tree pipit	*Anthus hodgsoni*	Passeriformes	Passeridae	RM	I, P
31.	Paddy field pipit	*Anthus rufulus*	Passeriformes	Passeridae	R	I, P
32.	Pied bush chat	*Saxicola caprata*	Passeriformes	Muscicapidae	R	I
33.	Red-vented bulbul	*Pycnonotus cafer*	Passeriformes	Pycnonotidae	R	I, P, F
34.	Red-wattled lapwing	*Vanellus indicus*	Ciconiiformes	Charadridae	R	I, SI
35.	Rose-ringed parakeet	*Psittacula krameri*	Psittaciformes	Psittacidae	R	F, P, G
36.	Rufous-backed shrike	*Lanius schach*	Passeriformes	Lanidae	R	I
37.	Small bee-eater	*Merops orientalis*	Coraciiformes	Meropidae	R	I
38.	White wagtail	*Motacilla alba*	Passeriformes	Passeridae	RM	I, SI
39.	Wire-tailed Swallow	*Hirundo smithii*	Passeriformes	Hirundinidae	R	I
40.	Yellow wagtail	*Motacilla flava*	Passeriformes	Passeridae	RM	I, SI

Status: R- Resident; RM- Resident Migrant; M- Migrant ; Food habit: I- Insectivorous; G- Granivorous; F- Fruits; P- Plants; SI- Small Invertebrates; SV- Small vertebrates; O-Omnivorous (Kler and Kumar, 2015)

Table 2. Relative abundance (%) of avian species- vs. phenological stages of mustard crop

S. No.	Birds	Sowing Stage		Seedling Stage		Vegetative Stage		Flowering Stage		Ripening Stage	
		L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
1.	Asian pied starling	5.69	3.98	3.99	4.86	5.58	2.77	5.40	10.17	-	-
2.	Bank myna	1.65	-	-	-	-	-	-	-	1.39	-
3.	Black drongo	6.86	7.79	5.91	3.23	4.11	4.98	-	9.46	4.92	2.08
4.	Black francolin	-	-	1.00	-	-	-	-	-	-	-
5.	Black ibis	-	-	1.90	-	-	-	-	-	-	-
6.	Black kite	-	-	4.59	3.05	4.86	3.16	-	-	2.97	2.11
7.	Black redstart	-	-	-	-	0.83	3.21	-	-	2.03	1.51
8.	Black-winged Stilt	-	-	-	-	1.15	4.38	-	-	-	6.88
9.	Blue rock pigeon	8.51	9.78	-	-	-	-	6.02	8.38	-	-
10.	Brain fever bird	-	-	0.66	-	-	-	-	-	-	-
11.	Cattle egret	-	-	-	-	5.09	-	7.78	8.16	2.40	2.17
12.	Common babbler	-	5.49	-	3.44	-	-	-	-	-	-
13.	Common hoopoe	-	-	1.24	2.81	0.98	2.05	-	-	-	4.51
14.	Common myna	20.08	11.85	14.01	10.06	14.12	9.14	12.67	12.60	16.03	4.39
15.	Common starling	-	-	-	-	1.63	-	-	-	-	-
16.	Common tailor bird	1.40	-	-	-	-	-	-	-	2.10	-
17.	Eurasian collared dove	5.88	3.74	7.43	5.25	-	5.54	7.30	8.38	6.90	10.30
18.	Greater coucal	-	-	-	-	1.44	1.61	-	-	-	-
19.	Greenish leaf-warbler	-	-	-	-	-	-	1.40	-	-	-
20.	Grey francolin	-	-	2.69	5.60	-	-	6.67	-	4.29	5.36
21.	Grey wagtail	9.34	9.14	3.98	5.99	5.49	4.54	2.33	-	-	-
22.	House crow	9.30	15.59	13.87	10.85	14.09	14.13	10.65	7.95	11.96	6.05
23.	House sparrow	-	-	-	-	-	-	-	-	1.60	1.94
24.	Indian peafowl	3.41	-	3.06	-	-	-	-	-	3.14	-
25.	Indian robin	-	-	-	-	-	-	-	-	-	4.31
26.	Indian roller	2.06	5.01	-	-	-	-	-	-	-	3.02
27.	Jungle babbler	1.40	-	2.03	-	-	-	4.71	9.45	-	-
28.	Large pied wagtail	-	-	5.02	7.13	6.62	4.76	1.18	-	-	-
29.	Oriental magpie robin	-	-	-	-	2.64	2.99	-	-	-	-
30.	Oriental tree pipit	-	-	-	-	-	-	1.18	-	-	-
31.	Paddy field pipit	3.41	8.35	1.17	2.68	3.16	4.10	2.07	7.66	-	-
32.	Pied bush chat	-	-	1.66	5.46	1.02	9.48	4.64	6.73	1.46	3.88
33.	Red-vented bulbul	-	-	1.52	3.20	-	-	-	6.44	3.18	-
34.	Red-wattled lapwing	7.42	7.79	7.17	2.94	6.40	2.05	4.58	4.58	6.54	3.36
35.	Rose-ringed parakeet	-	-	-	-	-	-	-	-	25.94	30.67
36.	Rufous-backed shrike	-	-	-	-	1.69	3.44	-	-	1.39	5.11
37.	Small bee-eater	-	-	-	-	-	-	1.18	-	-	-
38.	White Wagtail	12.32	11.46	13.95	17.65	13.89	11.52	10.86	-	-	-
39.	Wire-tailed Swallow	-	-	-	-	-	-	-	-	1.65	-
40.	Yellow Wagtail	-	-	3.15	5.80	5.20	6.15	3.36	-	-	-
Species richness		16	12	22	17	20	19	18	12	18	17
Shannon-Weiner Index		2.47	2.40	2.71	2.67	2.67	2.77	2.59	2.46	2.43	2.51
Species evenness		0.91	0.97	0.88	0.94	0.89	0.94	0.92	0.99	0.84	0.85

and Li (1992) had specified that bee-eaters, black drongos and white-breasted kingfishers are biological control specialists against white grub. Sahito et al. (2010) reported that avifaunal richness in *Brassica* genotypes such as gobhi sarson, raya and hyaola as 11, 13 and 16, correspondingly at the starting phase of siliquae formation in the Punjab. Sekercioglu (2006) highlighted the environment administrations furnished by birds for the management of pest in farming. Kler and Kumar (2015) reported that hemipterans as well as coleopterans make up the chief diet of insectivorous birds. Sekercioglu (2006) recommended that farm birds not really need to ingest considerable insect pest to have a substantial effect the population size. They figured out that 40% of the larva that protected from the attack of predators by ejecting food, passed on prior to changing into a butterfly, regardless of effectively enduring the first assault. Martin et al. (2013) led meta-investigation of hierarchical falls brought about by birds of prey and confirmed the discoveries that plants make the most of birds that eliminate their herbivorous all through a different environment as well as climatic regions. The present study has provided baseline data of avian species in mustard crop along with their foraging guilds. Higher relative abundance of insectivorous birds in mustard crop fields do have positive role in controlling insect pests.

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SPECIES DIVERSITY OF THRIPS ON COTTON

M Amutha* and R R Rachana[1]

ICAR-Central Institute for Cotton Research (CICR), Regional Station,
Coimbatore 641003, Tamil Nadu, India
[1]ICAR-National Bureau of Agricultural Insect Resources, Bengaluru 560024, Karnataka, India
*Email: amuento@yahoo.co.in (corresponding author)

ABSTRACT

Exploratory surveys were conducted to study the diversity of thrips species on cotton at Coimbatore, Tamil Nadu. Taxonomic studies revealed the presence of three species, viz. *Scirtothrips dorsalis* Hood, *Thrips palmi* Karny and *Thrips tabaci* Lindeman on leaves and four species, viz. *Thrips florum* Schmutz, *Thrips hawaiiensis* (Morgan), *Frankliniella schultzei* (Trybom) and *Thrips parvispinus* (Karny) in flowers. *T. parvispinus* is one of the notorious pest species of South East Asia and also a serious pest of quarantine importance. *F. schultzei, S. dorsalis*, *T. palmi* and *T. tabaci* are notorious pests as well as vectors of plant viruses. Since these species may attain major pest status, the report demands regular monitoring and surveys in cotton.

Key words: Cotton, thrips, diversity, leaves, flowers, *Scirtothrips dorsalis*, *Thrips palmi*, *T. tabaci*, *T. florum*, *T. hawaiiensis*, *T. parvispinus*, *Frankliniella schultzei*, diversity indices

India has the largest area under cotton and is also the largest producer of cotton. The area under cotton reached a high of 133.73 lakh ha with production of 365 lakh bales and productivity of 463.99 kg/ ha in 2019-20 (cotcorp.org.in). However, it is ranked 37th in the world in terms of productivity. Cotton plant is ravaged by multitude of sucking pests and there is a constant change in pest scenario. Among these sucking pests, the increased incidence of thrips, noted in recent years (Monga, 2021). A minor pest, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), has become a serious pest on Bt cotton in India (Sarode et al. 2009). Polyphagous nature, high reproductive capacity, short generation time, high survival of cryptic instars, reproduction by parthenogenesis, and development of resistance to insecticides, this insect became a serious pest of cotton in many cotton growing regions of India (Diaz Montano et al. 2012). In extreme cases, around 30-50% of lint yield loss has been reported (Cook et al., 2011). The world record indicates the occurrence of 6312 species (ThripsWiki, 2021). In India, a total of 739 species reported from 259 genera, of which 309 species belong to the suborder Terebrantia and 430 species belong to the suborder Tubulifera (Tyagi and Kumar, 2016). Losses caused by thrips to various agricultural and horticultural crops during the past decade, resulting in huge economic loss (Thrips Wiki, 2021). Several species of thrips are known to infest cotton. Therefore, the present investigation was undertaken to study the diversity of the thrips fauna on cotton at Coimbatore, Tamil Nadu.

MATERIALS AND METHODS

Thrips samples were collected from cotton growing areas of Coimbatore including experimental farm of ICAR-Central Institute for Cotton Research, Coimbatore (11°N77°E, 427.6 masl). Ten cotton plants were randomly selected for sampling. Cotton leaves and flowers were tapped on white paper then fallen thrips were collected in vials containing preservative media (9 parts 10% alcohol + 1 part glacial acetic acid + 1 ml Triton X-100 in 1000 ml of the mixture) (Bacci, 2008). The vials were labelled with host name, location and collection date for identification. Permanent slide mounts were prepared by following maceration and digestion protocol of Bhatti (1999) and were identified using appropriate morphological keys (Ananthakrishnan and Sen, 1980; Palmer et al., 1989) and they were observed through a Nikon Eclipse 80 i microscope and micro images were captured with a Nikon DS-ViI camera mounted on this microscope. Voucher specimens are deposited with ICAR-National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru, Karnataka, India. While examining the taxonomic identification, number of samples for each species also recorded to calculate Shannon Diversity Index.

RESULTS AND DISCUSSION

The results revealed seven thrips species, of which, *Scirtothrips dorsalis* Hood, *Thrips palmi* Karny and *T. tabaci* Lindeman were identified on leaves and four species, *T. florum* Schmutz, *T. hawaiiensis* (Morgan), *Frankliniella schultzei* (Trybom) and *T. parvispinus* (Karny) were from flowers. Diagnostic characters and keys for these are given below:

Frankliniella schultzei (Fig. 1): Ocellar setae pair III arising close together between anterior margins of hind ocelli and as long as side of ocellar triangle. Postocular setae pair IV as long as distance between hind ocelli. Pronotum with 5 pairs of major setae; anteromarginal setae slightly shorter than anteroangulars, one pair of minor setae present medially between posteromarginal submedian setae. Campaniform sensilla on metanotum absent. Posteromarginal comb on tergite VIII not developed. *Scirtothrips dorsalis* (Fig. 2): Body yellow with median brown marking on tergites III–VII . Postocular and ocellar region closely striate. Ocellar setae pair III arise between posterior ocelli, well behind tangent between their anterior margins. Two pairs of post-ocellar setae as long as ocellar setae pair III. Pronotum striate closely, posteromarginal setae S2 longer than S1. *Thrips florum* (Fig. 4): Head with ocellar setae III outside ocellar triangle. Postocular seta II very much smaller than I or III. Mesonotum without sculpture lines close to anterior campaniform sensilla. Clavus with subapical seta longer than apical seta. Sternites III–VII with 6–14 discal setae. *Thrips hawaiiensis* (Fig. 3): Head with ocellar setae III outside ocellar triangle. Postocular setae I and II subequal. Mesonotum with sculpture lines close to anterior campaniform sensilla. Clavus with apical seta longer than subapical seta. Sternites III–VII with 12–25 distal setae. *Thrips palmi* (Fig. 5): Ocellar setae pair III small, arising outside ocellar triangle and postocular setae pair I slightly longer than ocellar setae III. Metanotum with irregular longitudinal lines converging to posterior margin, with anteriorly curving transverse lines; median setae arising well behind anterior margin, campaniform sensilla present. Forewing first vein with 3 distal setae. Abdominal tergite II with 4 marginal setae laterally; tergite VIII with complete comb. *Thrips parvispinus* (Fig. 6): Ocellar setae pair III at the anterior margin of ocellar triangle; postocular setae III shorter than postocular setae I and IV. Metanotum with median reticulations; median setae placed well behind the anterior margin; campaniform sensilla absent. First and second veins of fore wing with continuous setal row. Posterior margin of abdominal tergite VIII without comb. Abdominal sternites III–VI with discal setae, but absent on II and VII. *Thrips tabaci* (Fig. 7): Abdominal pleurotergites with closely spaced rows of regular, fine microtrichia; lateral margins of tergites with microtrichia on sculpture lines; tergite IX with one pair of campaniform sensilla, anterior pair absent; antennal segment V not sharply paler than IV.

Key to species

1. Antennae 8-segmented (Fig. 9).....**2**
- Antennae 7-segmented (Fig. 8).....**3**
2. Abdominal tergites with lateral side completely covered with many microtrichia (Fig. 16); forewing second vein with irregular setal row (Fig. 17)..... ..... ***Scirtothrips dorsalis* Hood**
- Abdominal tergites without microtrichia covering lateral side; forewing second vein uniform setal row (Fig. 12)..... ***Frankliniella schultzei* (Trybom)**
3. Abdominal sternites without discal setae..... .....**4**
- Abdominal sternites with discal setae..... .....**5**
4. Abdominal pleurotergites with several rows of fine ciliate microtrichia; metanotal sculpture with median reticulations (Fig. 10), campaniform sensilla absent.... .....***Thrips tabaci* Lindeman**
- Abdominal pleurotergites without fine ciliate microtrichia; metanotal sculpture longitudinally striate (Fig. 11), campaniform sensilla present ....***Thrips palmi* Karny**
5. Abdominal sternite VII without discal setae (Fig. 19) ..... ***Thrips parvispinus* (Karny)**
- Abdominal sternite VII with discal setae..... .....**6**
6. Mesonotum sculptured around campaniform sensilla near anterior margin (Fig. 14); clavus with apical seta longer than subapical seta (Fig. 13)..... .....***Thrips hawaiiensis* (Morgan)**
- Mesonotum not sculptured around campaniform sensilla near anterior margin (Fig. 15); forewing clavus with apical seta shorter than subapical seta (Fig. 18)..... ***Thrips florum* Schmutz**

The survey represents comprehensive documentation of thrips species on cotton at Coimbatore, Tamil Nadu.

S. dorsalis, *T. palmi* and *T. tabaci* were present on leaves. Whereas, *T. florum*, *T. hawaiiensis*, *F. schultzei* and *T. parvispinus* were recorded in flowers. Earlier reports documented *S. dorsalis*, *T. palmi* and *T. tabaci* in cotton ecosystem (Rajendran et al., 2018; Senguttuvan, 2019). Thrips florum breeds in flowers of a wide range of plants and causes direct damage by puncturing flowers. The species causes considerable damage in the bud condition, with the result that the flowers become smaller, the petals shrink and show feeding scars (Ananthakrishnan 1971). This is one of the most common and widespread flower thrips across Asia to the Pacific islands. On cotton, Akram et al. (2003) reported from Pakistan. *T. hawaiiensis* is also a common thrips species of various flowers. It affects the crop during vegetative growing stage, flowering stage and fruiting stage. *F. schultzei* is one of the serious polyphagous pests among the genus *Frankliniella*. It causes economic damage to various ornamental and vegetable crops. *T. parvispinus*, which is designated as

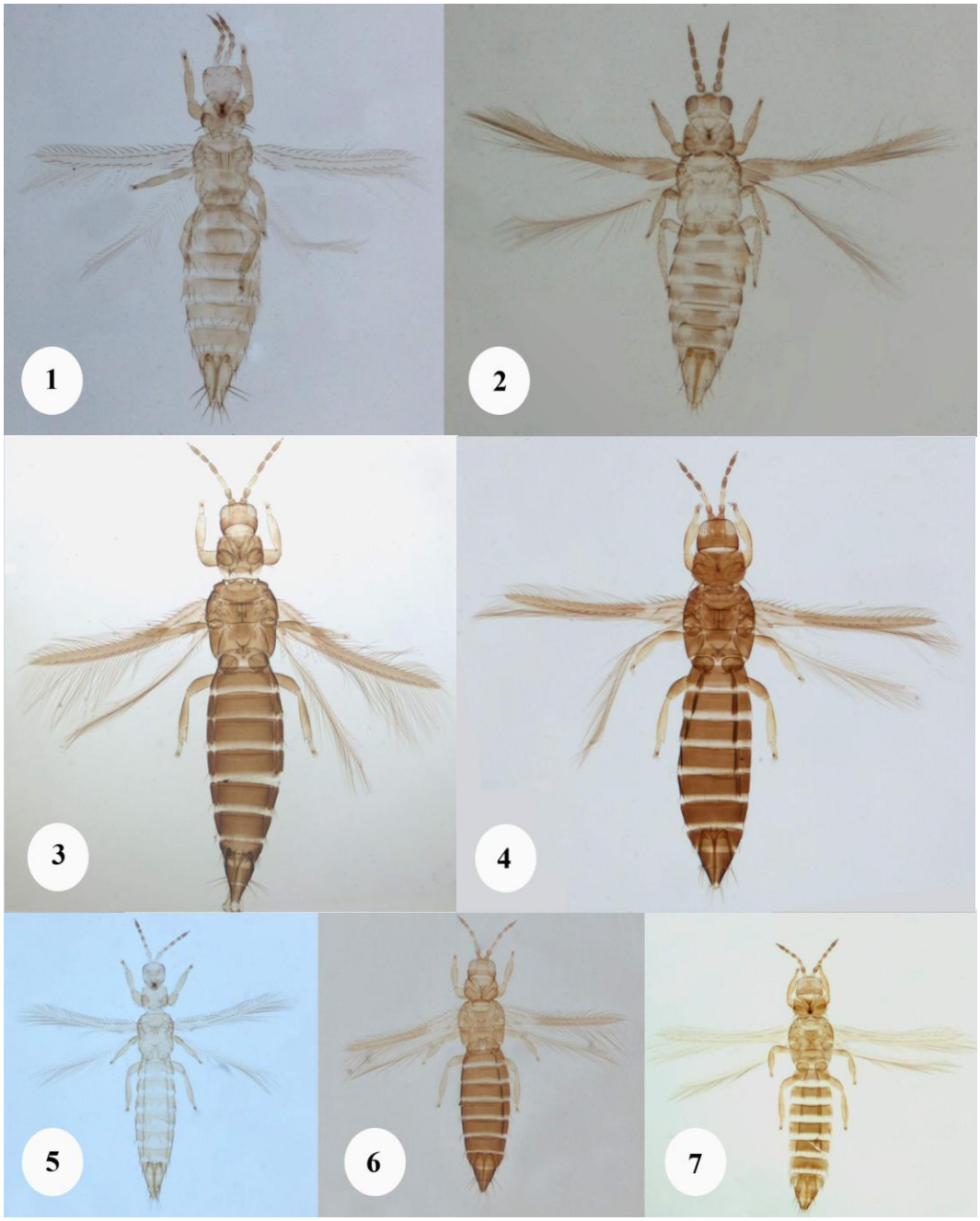

Fig. 1-7. (1) *F. schultzei*; (2) *S. dorsalis*; (3) *T. hawaiiensis*; (4) *T. florum*; (5) *T. palmi*; (6) *T. parvispinus*; (7) *T. tabaci*

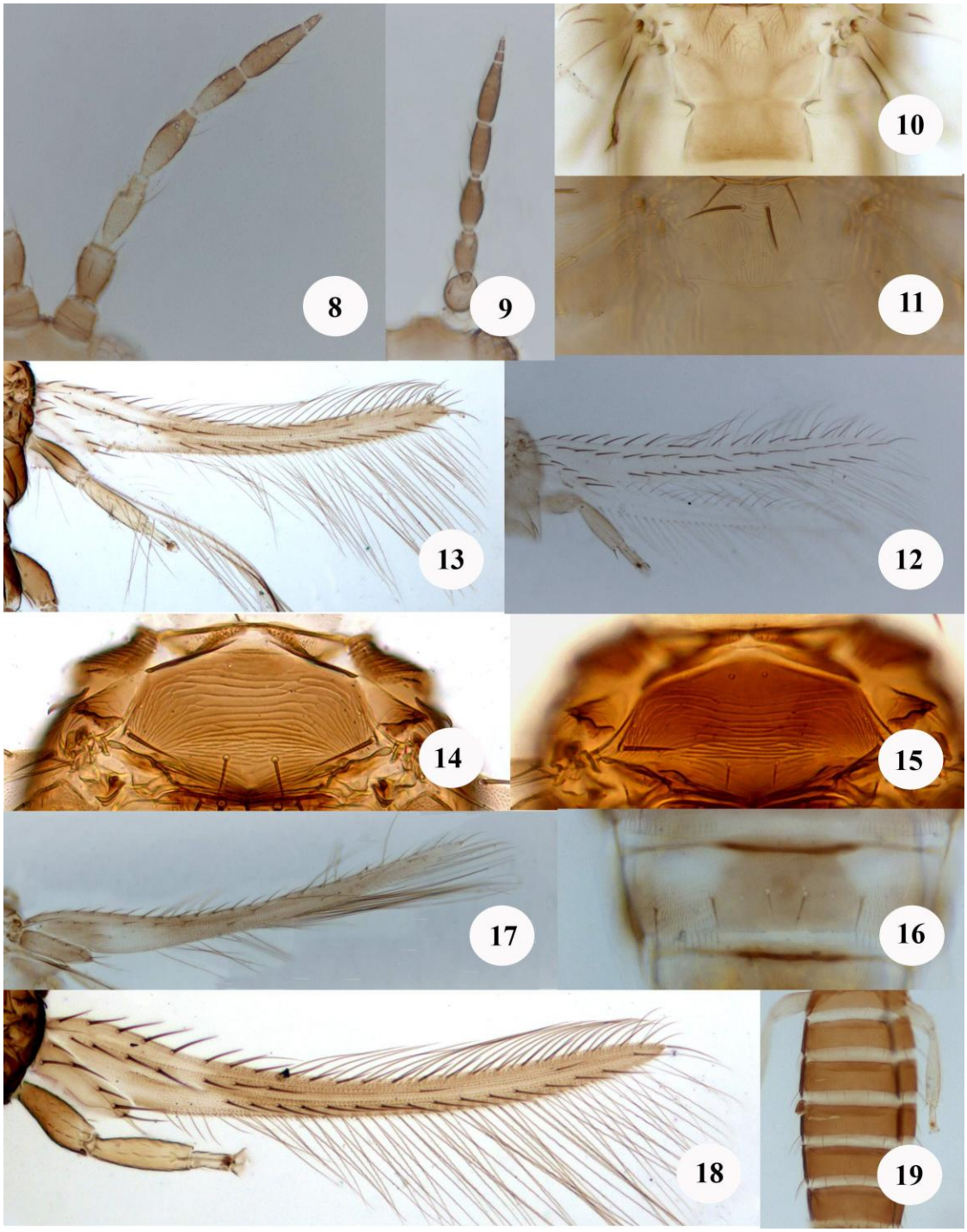

Fig. 8-19. (8) *T. parvispinus*, antenna; (9) *S. dorsalis*, antenna; (10) *T. tabaci*, metanotum; (11) *T. palmi*, metanotum; (12) *F. schultzei*, forewing; (13) *T. hawaiiensis*, forewing; (14) *T. hawaiiensis*, mesonotum; (15) *T. florum*, mesonotum; (16) *S. dorsalis*, abdominal tergite VII; (17) *S. dorsalis*, forewing; (18) *T. florum*, forewing; (19) *T. parvispinus*, abdominal sternites II-VII

Table 1. Shannon diversity index of thrips in cotton

Species	No. of individuals	Pi	In Pi	Pi In Pi
Scirtothrips dorsalis	28	0.107	-2.23	-0.239
Thrips palmi	19	0.072	-2.63	-0.189
T. tabaci	12	0.046	-3.08	-0.142
Thrips florum	109	0.414	-0.88	-0.365
Thrips hawaiiensis	89	0.338	-1.08	-0.367
Thrips parvispinus	4	0.015	-4.20	-0.063
Frankliniella schultzei	2	0.008	-4.74	-0.038
Total	263	1		

Shannon Index (H) = 1.403, Pi -Proportion of individuals

one of the notorious pest species of South East Asia, is a serious pest on numerous agricultural and horticultural crops. Occurrence of this species in India has been first reported on papaya from Bangalore (Tyagi et al., 2015). Later, on flowers of *Dahlia rosea* in Puttur, Karnataka (Rachana et al., 2018). This is the first record of *T. parvispinus* on cotton in India, which is already known for its quarantine importance. Based on the results of Shannon diversity index, *T. florum* followed by *T. hawaiiensis* found to be dominant in flowers, *S. dorsalis* found to be dominant in leaves (Table 1). Based on the dominance of thrips species, pest management practices need to be formulated. In this study seven thrips species were recorded on cotton. *T. parvispinus* is one of the notorious pest species of South East Asia and also a serious pest of quarantine importance. *F. schultzei*, *S. dorsalis*, *T. palmi* and *T. tabaci* are notorious pests as well as vectors of plant viruses. Since these species may attain major pest status, the report demands regular monitoring and surveys for them on cotton.

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BIOLOGY OF *XYLOTRECHUS BASIFULIGINOSUS* HELLER- A BORER OF KHARSU OAK TREES IN THE WESTERN HIMALAYA

Gaurav Chand Ramola* and Arun Pratap Singh

Entomology Branch, Forest Protection Division, Forest Research Institute,
Dehradun 248006, Uttarakhand, India
*Email: gauravramola30@gmail.com (corresponding author)

ABSTRACT

Biology of *Xylotrechus basifuliginosus* (Cerambycidae: Coleoptera: Clytini) was studied on its host Kharsu oak *Quercus semecarpifolia* Smith. It has an annual lifecycle with five larval instars. Beetles emerge at the onset of the rainy season (June-July) under natural conditions at 2600-2800 m. Females soon after copulation lay up to 34 eggs in cervices and covered depressions on the bark of oak trees. The larval period is 269 days under natural conditions. The growing larvae feed in the sapwood up to December and thereafter go into hibernation (end of December-mid of March) in larval galleries. Pupation is triggered by sudden rise in temperature in spring (end of March), pupal period is ~94 days. The beetles emerge from the pupal chamber by chewing the bark and making a circular exit-hole (5 mm dia). The adult morphology (both sexes) and male genitalia, are described and compared with its congeners *X. smei* and *X. stebbingi* also known from northern India, besides the morphology of egg, larval and pupal stages.

Key words: *Xylotrechus basifuliginosus*, Cerambycidae, *Quercus semecarpifolia*, Garhwal, stem borer, oak, male genitalia, seasonality, lifecycle, moist temperate forest, feeding pattern.

The genus *Xylotrechus* is Holarctic in origin and richest genus in Southeast Asia (Cherepanov, 1988) with at least 180 species of the genus *Xylotrechus* known across the globe (Ohbayashi and Niisato, 2007). The adults of this genus generally have robust form, antennae are filiform and less than the half of body length, elytra are generally tapered or rounded and femora are not distinctly spined. The larval habits and host plants preferences of *Xylotrechus* spp. are various and the major hosts include the genera *Quercus* (*Q. semecarpifolia, Q. leucotrichophora*), *Picea smithiana, Pinus* sp. and *Salix* spp. (Beeson, 1941; Linsley, 1964; Mathur and Singh, 1959). Beeson and Bhatia (1939) described the biology of certain species of genus *Xylotrechus* in Indian forest records. *X. basifuliginosus* is a black coloured beetle (15 mm), which inhabits moist temperate forests of Chakrata hills (Dehradun district) in Garhwal region of Uttarakhand state and Chopal (Shimla) and Dharamsala (Kangra) both in Himachal Pradesh state, in the Indian Western Himalaya between ~1500-3000 m. This species is a mainly borer of kharsu oak (*Quercus semecarpifolia*) which not only causes tree mortality in Western Himalaya (Singh, 2011; Kariyanna *et al*., 2017) but also considerably deteriorates the quality of timber and wood damaged by larvae is rendered totally useless for commercial purposes and hence preparing marketable timber fraught with difficulties. Previously Singh (2011) identified this species as a secondary borer causing damage to kharsu oak in Chakrata Forest Division, Uttarakhand. K.M. Heller firstly described the morphology of adults of *X. basifuliginosus* in 1926. However, the morphology of remaining life stages of eggs, larva, and pupa have yet to be described, so this study was undertaken to fill this gap. As a result, this study will serve as a foundation for interpreting and comprehending other parts of this borer's biology, as well as assisting in the management and control of this borer in the Western Himalayan region.

MATERIALS AND METHODS

The present study was carried out from 2018-2020, in the Deoban Reserve Forest (N 30.74806; E 77.86639; 2606-2815m) of the Chakrata Forest Division, Dehradun district, Uttarakhand state in India. In order to study the biology of *X. basifuliginosus*, borer infested kharsu standing and fallen trees and logs, were marked and studied. 3-5 borer infested branches of fallen and dead kharsu oak trees were cut into logs in fortnight and chopped into pieces to record the number the larval stages and their duration period up to the formation of pupa. While during emergence in June-July in field condition, observations were taken after every week. Data was recorded on the number

and sex of the emerging beetles, copulation, mating and egg laying.

Morphological studies on *X. basifuliginosus* eggs, larvae, pupa and adults were conducted in the Forest Research Institute laboratory in Dehradun, Uttarakhand, India. Digimizer 4.0 image software and Olympus SZX16 stereoscopic microscope were used to study and measure the morphology of different developmental stages. The width of head capsules of different larval instars were measured with ocular micrometer. Final instar larva was described for the morphological study. For morphological studies of adults, the emerged adults were preserved by treating with 10% KOH for overnight to soften the chitin and dissolve the soft parts. Specimen thus treated was kept in clove oil and permanent mount in Canada balsam were prepared. The length, width and other morphological character of pupa was also studied under microscope. Besides two congeners (*X. smei* and *X. stebbing*) of *X. basifuliginosus* that also occur in the Western Himalaya having overlapping distributional ranges were compared morphologically with *X. basifuliginosus*. Male genitalia of *X. basifuliginosus* and *X. smei* were studied by following Ehara (1954) method and compared with four Japanese *Xylotrechus* spp.

RESULTS AND DISCUSSION

Biology

Emergence of beetles occurs during June and July when the mean temperature and relative humidity are above 22°C and 59%, respectively. Mating takes place just after the emergence during day time, with peak time being 11:00-15:00 hr on the stems on which they emerged or on the foliage exposed to bright sunlight. Copulation lasts for about 8 to 10 min with repeated copulation by the same pair. While at rest the females stretch out their ovipositors and twist them backwards and forwards for up to an hour. The next day after mating oviposition takes place in June- July. The female after mating moves continuously on the Kharsu stems searching with its ovipositor by stretching it with full length for suitable cracks and crevices in the bark of stem to lay eggs. Eggs are partially exposed to exterior and partially inserted in the bark. According to Shylesha (1992), in *Xylotrechus quadripes* under normal conditions each female deposits one or two eggs at a time but 1 to 10 eggs have been noticed by Ramaiah (1983). The maximum number of 34 eggs were laid within 24 hr during June (25.1°C, 56% RH) on the first day after emergence and the number of eggs laid steadily decline to thereafter to 2 on the 8th day after emergence. A total of 32 eggs ($\bar{x}$) are laid by single female in its lifetime whereas a total of 28 eggs were counted in single virgin female (Fig. 1a,b). Overall hatching % was 78.71%. Hatching of eggs takes 7 days in summer (June; 23.7°C, 63% RH) at Deoban, Chakrata Forest Division, Uttarakhand).

Fig. 1. Eggs inside the abdomen of a virgin female

Duration of first instar larval stage is 24-29 days (27.1 days in July). Second instar larval duration is 36-44 days (41.6 days in August-September) whereas third instar lasts for 49-57 days (56.4 days in September-October). Fourth instar larval stage duration is 66-71 days (69.9 days in November-December). 5th instar larval stage duration is 71-76 days (74.6 days from December-March). Fifth instar larva feeds in the sapwood and go into hibernation from end of December to March inside the larval gallery (2.2-10.1°C, 40-66.8%RH). With the onset of warm weather, larva becomes active and starts boring in the wood from mid-March of the following year.

Pupa takes 81-97 (94 days) with the callow adult taking 6-7 days to complete sclerotization and remains in the chamber for 3-4 days before emergence. The emergence of beetles of the next generation takes place during June-July of the following year. Adults chew a 5mm dia exit hole in the bark once the larvae have completed their development. Overall, the mean male and female ratio of 3 years was 1.7:1. The mean data of longevity of 3 years indicated that the females live moderately longer (12.53± 1.92 days) than male adults (10.06± 2.28 days).

Taxonomy and morphology

The freshly laid eggs are elongate, elliptical in shape and milky white and one end more acutely pointed than the other (Fig. 2). Later the eggs become slightly yellowish and bloated (Fig. 3). The eggs measured 1.84± 0.05 mm long and 0.59± 0.06 mm wide. 1st instar larval length varies between 1.83-1.96 mm and width from 0.36-0.52 mm and whitish; while 2nd instar

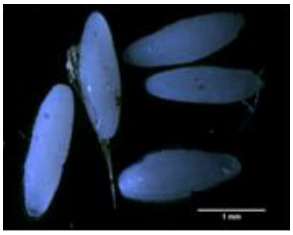

Fig. 2. Eggs

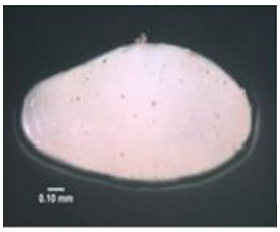

Fig. 3 Egg before hatching

measured 3.80-4.41x1.10-2.00 mm, with colour being pale to whitish-yellow, robust and thick; 3rd instar measured 5.0-9.20x 2.8-3.91 mm and yellow, while 4th instar larva is cylindrical, winkled and yellow, measuring 12.0-17.2x 4.0-5.0 mm; 5th instar was 19.4-24x 4.50-5.50 mm and yellow; all are apodous. The width of head capsules ranges from 0.61± 0.07 to 4.16± 0.11 from I to V larval instars. Just before pupation, the larva contracts, causing the intersegment skin to wrinkle and the abdominal segment to progressively taper, with the tapering most noticeable in the 4th and 5th segments. The 5th instar has its head trapezoidal, glabrous, depressed, and creamish with rounded edges; its mouth frames are dark brown and strongly pigmented (Fig. 4c,d), and mandibles black, short, robust, and have gouge-like cutting edges with a dark brown basal apical portion and a row of setae (Fig. 4e). Head capsule is with occipital foramen which is divided into small anterior and large posterior portion by tentorial bridge. Prothorax is thick with yellow pigmentation having distinct proalar plates and short hairs on the lateral region. The dorsal surface consist of large, rectangular, sclerotised plate called pronotum which is anteriorly shinning, glabrous or velvety pubescent. Abdomen ten-segmented, with its first six to seven segments of tergal and sternal areas similar in appearance; these usually have rounded structure called 'ampullae' which are broad, fleshy oval protuberance and are parallel in dorsal and ventral surface (Fig. 4a,b). These help larva in moving along inside the gallery. The anal region is trilobite with short fine hairs (Fig. 4f).

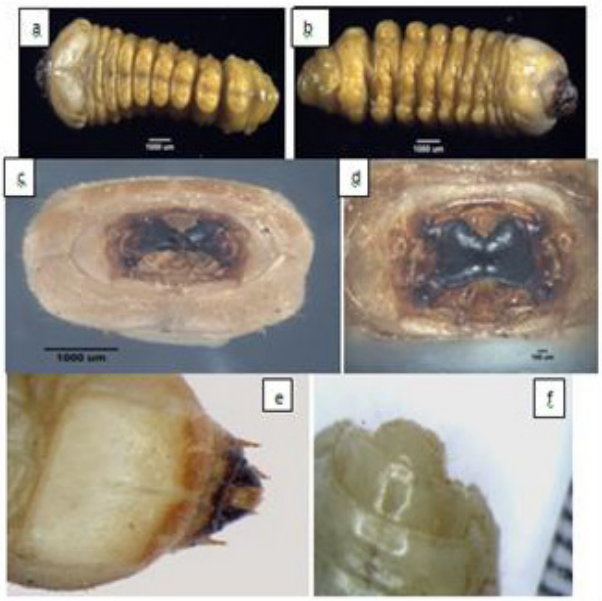

Fig. 4. V instar larva of *X. basifuliginosus* (a) Ventral view (b) Lateral view (c) Frontal view (d) Mouth parts (e) Ventral view of mouth parts (f) Last abdominal segments

Pupa is exarate type, closely resembling adult both in shape and size; and measuring 17.75± 0.95 mm long and 5.25± 0.50 mm wide, yellow, with head round, abruptly hooked at their apex and above carinate along the cutter side, pubescent at the base, labrum longitudinally striate, with short recumbent setae, clypeus transversally striate, pubescent at sides, forehead with V-shaped carina, the tip of which reaches the level of lower margin of the eyes and the ends exceed the level of antennal supports forming an oval-elongate flat tubercle and reaches the hind margin of the head (Fig. 5a). Antennae are pale yellowish in colour, short, and situated on the lateral side of the body, reaching up to the metanotum where they terminate above the elytra and wing (Fig. 5b). Mesonotum and metanotum are glabrous, shining and have a pronounced scutellar groove (Fig. 5e). The abdomen is fairly elongate, inflated, regularly narrowed and bowed towards the tip, widest at segment IV; sternites lack spinules, whereas 1st-7th tergites have several sparsely acicular black and short spinules (Fig. 6d); spines are stout, more strongly curved on the 7th to 9th abdominal tergites (Fig. 5c, d). 6th-7th acicular spinules are transversely arranged along

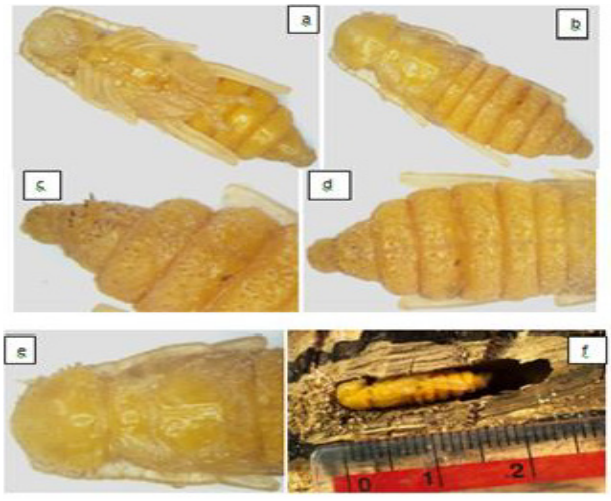

Fig. 5. Pupa of *X. basifuliginosus* (a) Ventral view (b) Dorsal view (c) Tergite with spinules (d) Abdominal tergites with spines (e) Dorsal view of pronotum and thorax (f) Pupa inside pupal chamber

the centre and 5th acicular spine is transversely disposed along the hind margin. The apex of the 8th tergite is somewhat inflated, with four tiny spines towards the rear border. The pupal chamber in 38 mm long, 6 mm wide, free of frass and formed at a short depth in the sapwood (Fig. 5f).

Heller described the adult *X. basifuliginosus* in 1926, mainly by comparing it with the genus *Perissus* (Cerambycidae: Clytini) i.e. *P. quercus* occurring in the same area with which it was perceived by him to be confused. Heller's (Heller, 1926) morphological description of *X. basifuliginosus* is not comprehensive and difficult to interpret because of the terminology used. The distinctive characters include: colour varies from black to dark brown with grey pubescence on the head, thorax and abdomen region. Elytra are dark brown and covered with grey pubescence, with three creamish coloured transverse bands in male, while these bands are yellow in female (Fig. 6, 7). These bands are present on the subbasal, median and sub-apical area of elytra, while the elytra is tapered at the end. Mandibles are triangular, black, short, pointed and posses short brown setae. Head and pronotum is seperated by pale band. Antennae is 11 segmented in both the sexes, filiform type, black in male and brown coloured in female, inserted in the middle of the frons and measures less than the half of the body length. The antenna are attached to the head capsule by a large basal membrane which is often regarded as the 'basal segment'. Pronotum is dark brown colour and densely covered with brownish pubescence. Abdominal sternites are densely covered with white and yellow alternate bands in female and white-black bands in males which are covered with long erect hairs. Legs are hairy, dark brown in colour, strong and moderate size. Femur of foreleg and midleg is stout and longer than tibia. Shape of hind leg is slender and slightly shorter than tibia. Fore, mid and hind tibia have tibial spurs. Spur of hind tibia is long. Tarsus is four segmented. First meta-tarsomere is longer than the total length of remaining tarsomere and twice as long as 2nd and 3rd combined of remaining tarsomeres. The last tarsal segment has pair of claws. Hind femora nearly reaches but does not exceed the elytral apics.

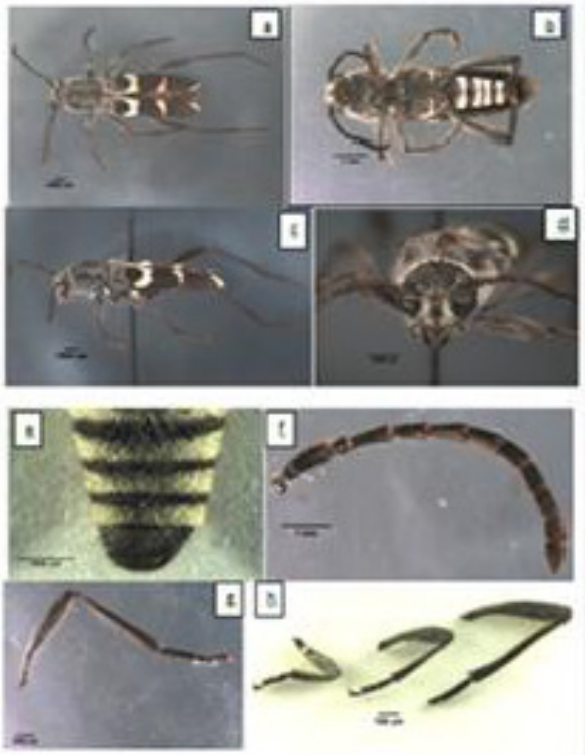

Fig. 6. Male *X. basifuliginosus* (a) Dorsal view (b) Ventral view (c) Lateral view (d) Frontal view (e) Abdominal sternites (f) Antennae (g) Hindleg (h) Foreleg, Midleg and Hindleg

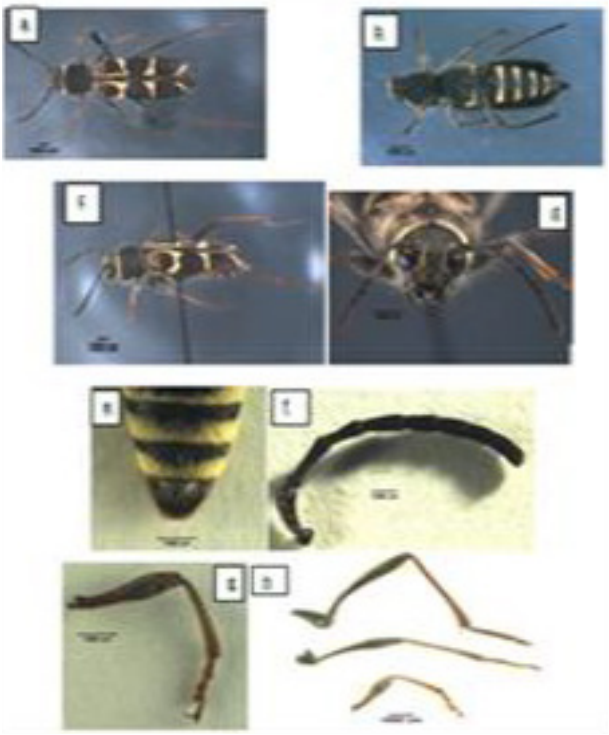

Fig. 7. Female *X. basifuliginosus* (a) Dorsal view (b) Ventral view (c) Lateral view (d) Frontal view (e) Abdominal sternites (f) Antennae (g) Hindleg (h) Foreleg, Midleg and Hindleg

A comparison of adults of *X. basifulginiosus* with congeners *X. smei* and *X. stebbingi* revealed the following: these are distinguished by the body length, spots on pronotum, colour pattern on elytra, prothorax and underside of the abdomen (Fig. 8); *X. stebbingi* is largest (12-18 mm) followed by *X. basifuliginosus* (10.5-19 mm) and *X. smei* (10-17mm), respectively in

body length. Pronotum is without spots on the disc in *X. basifuliginous* (Fig. 8a) while *X. smei* has two spots (Fig. 8b) and *X. stebbingi* has four spots (Fig. 8c). *X. basifuliginous* has three distinct creamish transverse bands on the elytra, while the size of the three bands are narrow and short in *X. smei* and are interrupted and broken in *X. stebbingi*. Prothorax of *X. basifuliginosus* is black, reddish in *X. smei* and grey with brown spots in *X. stebbingi*. While on the ventral side of abdomen these three species show variation in the transverse bands which are alternately white and black in *X. basifuliginosus* , pale coloured spots or bands in *X. smei* and are ashy white spots in *X. stebbingi* instead of bands. Legs and antennae are black and densely covered with grey hairs in male of *X. basifuliginosus* whereas it is reddish brown and densely covered with grey hairs in male of *X. stebbingi* and *X. smei*.

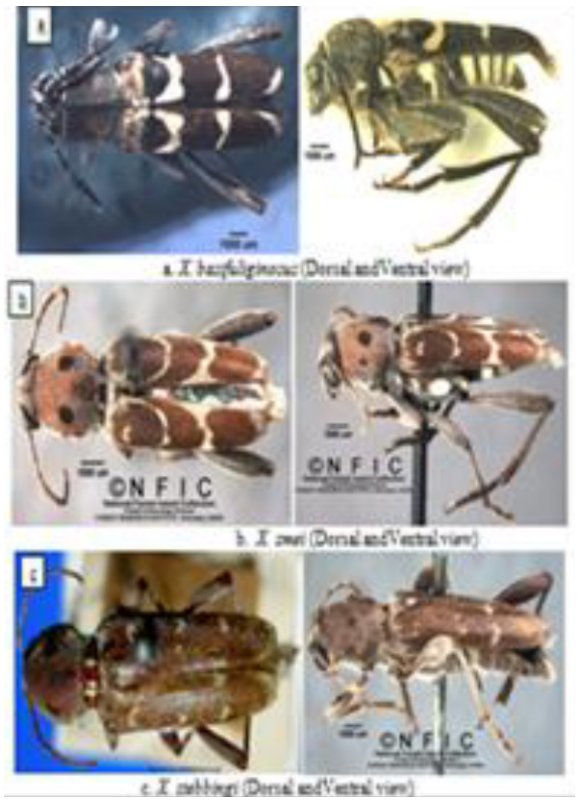

Fig. 8. Comparison of morphology of adult of three species of *Xylotrechus* (a) *X. basifuliginosus* (b) *X. smei* (c) *X. stebbingi*

Male genitalia- *X. basifuliginosus* vs. *X. smei;* In *X. basifuliginosus* the tegmen (Fig. 9a) measures 1.5x 0.98, with lateral lobes 0.13x 0.08 mm, narrowest at the apical and gradually constricted towards the apex, sparsely covered with short and fine brown setae near apex, with long and thick dark brown setae 0.49 mm long apically' tegmental ring 0.96x 0.58 mm, wide and slightly narrow or V- shaped towards the base; lateral

Fig. 9. Male genital parts of *X. basifuliginosus* (a) Tegmen (b) Median lobe (c) 8th Sternite (d) 8th Tergite

lobes smaller than the roof and apex is rounded. Median lobe (Fig. 9b) is slightly curved, relatively long, 2.08x 0.49 mm, with median orifice slightly projected or pointed, exposing the reflexed apical part of the ventral plate. Median struts are slightly curved and longer than median lobes and separated from each other. 8th sternite (Fig. 9c) measures 0.67x 0.98 mm wide, semicircular, densely covered with long brown setae present at apical half. Spiculum gastrale 1.23 mm long, Y-shaped, slender and longer than the 8th sternite. 8th tergite (Fig. 9d) 1.43x 1.26 mm, densely covered with short and brown-coloured setae which are long apically. In contrast, *X. smei* the tegmen is 1.44x 0.61 mm (Fig. 10a); lateral

Fig. 10. Male genital parts of *X. smei* (a) Tegmen (b) 8th Tergite (c) 8th Sternite (d) Median lobe (e) Spiculum gastrale

lobes 0.15x 0.08 mm, narrowest at the apical gradually constricted towards the apex, covered with short and fine brown setae near apex, with long and thick dark brown, 0.34 mm long setae apically. Tegmental ring 0.65x 0.48 mm, somewhat U-shaped towards the base. Lateral lobes larger than the roof and apex is rounded. Median lobe 1.83x 0.45 mm, strongly curved with median orifice slightly projected or pointed, exposing the reflexed apical part of the ventral plate (Fig. 10d). Median struts strongly curved and longer than median lobes and separated from each other. 8th tergite 1.25x 1.14 mm wide, sparsely covered with short and brown coloured setae (Fig. 10b). 8th sternite (Fig. 10c) 0.97x 1.9 mm, semicircular, sparsely covered with long brown setae present at apical half. Spiculum gastrale 1.38 mm long, Y-shaped, slender and longer than the 8th sternite (Fig. 10e).

Ehara (1954) studied the male genitalia of four Japanese spp. of genus *Xylotrechus* (*X. chinensis, X. clarinus, X. cuneipennis* and *X. pyrrhoderus*) and concluded that these differ in median lobe. Five genitalia characters (length, curvature of median lobe with median struts, length between median lobe and median struts, structure of edge of median orifice and length between lateral lobes and roof) were selected as given in Ehara (1954). These were analysed in 6 species (*X. basifuliginosus, X. smei* and four Japanese spp.). These analyses revealed that length of male genitalia of *X. basifuliginosus* is largest (3.39 mm) amongst all; curvature of median lobe with median struts, it is slightly curved in *X. basifuliginosus* and *X. chinensis, X cuneipennis* and *X. pyrrhoderus* whereas it is moderately curved in *X. clarinus* and strongly curved in *X. smei*; structure of edge of median orifice is slightly projected in *X. basifuliginosus* and *X. smei* whereas in Japenese species it is weakly projected; and length between lateral lobe and roof, lateral lobe is smaller in *X. basifuliginosus, X. chinensis* and *X. pyrrhoderus* whereas it is larger than roof in *X. smei, X. clarinus* and *X. cuneipennis*.

The lifecycle of *X. basifuliginosus* was found to be different in some respect from *X. stebbingi* which also attacks other Western Himalayan oaks i.e. *Q. leucotrichophora* and *Q. floribunda* growing in the vicinity of *Q. semecarpifolia* but separated along the altitudinal gradient. *Q. semecarpifolia* is timberline species while the latter two occur at lower altitudes in the temperate zone. *X. stebbingi* which infests *Q. floribunda* oak logs and stumps, the beetles emerge in June-July and has an annual life cycle (Beeson and Bhatia, 1939) whereas according to Stebbing (1914), the larval stage of *X. stebbingi* is about 270 days, however the pupal stage lasts from 42 days to 2 months on *Q. floribunda* and emergence takes place at the end of July or August in north west Himalaya while in *X. basifuliginosus* pupal duration ranges from 81-97 days during March-May. *X. smei* is also another congener of *X. basifuliginosus* which attacks *Q. leucotrichophora*. The longest oviposition period is 6 days in April. Eggs are laid in crevices and covered depressions on the surface of bark in large clusters and egg hatches in 4-5 days in the months of April. The shortest larval period of *X. smei* on sal logs (*Shorea robusta*) is 52 days in April-May and 18-19 days of the pupal period in Dehradun (Beeson and Bhatia, 1939). The shortest life cycle of *X. smei* overwintering broods is about 6 months and the longest might be 16 months (Beeson and Bhatia, 1939). Emergence of *X. smei* (in Dehradun) begins at the end of March from overwintered broods and is at its peak in May-June and continues to the end of the November (Beeson, 1941).

Male genitalia of Cerambycinae are distinct from the other subfamilies of Cerambycidae by having quite well developed 'roof part' while 'lateral lobes' shows variation within the groups (Sabanoglu and Sert, 2018). The length of male genitalia of *X. basifuliginosus* is largest amongst all. The main difference amongst *Xylotrechus* spp. is in the length of 'lateral lobes', and 'roof' of tegmen which is the highly variable in shape in this species and enumerated as a valuable character for differentiation in this species. Cerambycid borers are one of the main factors responsible for the mortality and decline of *Q. semecarpifolia* oak trees in the temperate zone of the western Himalaya and *X. basifuliginosus* also deteriorates the quality of wood and timber. Therefore, understanding the lifecycle of this stem borer in this intricately balanced oak forest ecosystem can help us in better conservation and management of Kharsu oak forests.

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AUTHORS CONTRIBUTION

GCR and APS conceived and designed research; GCR gathered data and conducted experiments under guidance of APS; GCR and APS analyzed data; GCR prepared draft of manuscript and A.P.S. reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest in preparing this manuscript.

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RESISTANCE IN *SPODOPTERA LITURA* (F.) TO INSECTICIDES AND DETOXIFICATION ENZYMES

S Ramesh Babu* and Beerendra Singh

Department of Entomology, Rajasthan College of Agriculture,
MPUAT, Udaipur 313001, Rajasthan, India
*Email: babuento2018@gmail.com (corresponding author)

ABSTRACT

Standard leaf dip bioassay experiments were conducted to study the insecticide resistance in *Spodoptera litura* (F.) in soybean. Chlorantraniliprole was the most toxic while, cypermethrin and organophosphates (profenophos and triazophos) recorded least toxicity against *S. litura* populations. Field population of *S. litura* showed the highest resistance to cypermethrin with resistance ratios (RRs) ranging from 244 to 376. The field populations collected from three locations of Southern Rajasthan exhibited low resistance to newer insecticides, chlorantraniliprole and spinetoram with Lab-SS strain. All the enzyme activities were significantly higher in the whole larval extracts and midgut extracts of *S. litura* larvae when compared to the Lab-SS strain. Analysis of the carboxyl esterase enzyme in the native PAGE assay revealed that the electrophoretic profiles showed presence of different band regions (EST 1 to EST 7) with esterase activity in the field populations along with the laboratory susceptible strain. The susceptibility of three field populations to different group of insecticides along with the profile of detoxification enzymes indicate the need to formulate a region-specific insecticide resistance management (IRM).

Key words: *Spodoptera litura*, carboxylesterase, detoxification enzymes, insecticide resistance, electrophoresis, resistance ratio, chlorantraniliprole, cypermethrin, organophosphates

Larvae from the *Spodoptera* genus are distributed worldwide and gaining importance due to their polyphagous nature and causing significant losses in economically important crops. *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is commonly known as the common cutworm and one of the most important polyphagous pests of soybean. Larvae feed on the foliage, resulting in complete defoliation and in case of severe infestation, complete devastation of soybean crop occurs during its reproductive stage (Chattopadhyay et al., 2019). In recent years, the tobacco caterpillar has become a serious pest on soybean in some parts of India (Dhaliwal et al., 2010; Chattopadhyay et al., 2019). Indiscriminate use of different groups of insecticides for the control of *S. litura* led to the development of multiple types of resistance (Armes et al., 1997; Kranthi et al., 2001; Ahmad et al., 2007a,b). The extensive use of conventional insecticides belonging to organochlorines, carbamates, organophosphates (OPs) and pyrethroids) and other newer group of chemicals like fipronil, avermectins, indoxacarb, spinosad, emamectin benzoate, chlorantraniliprole and insect growth regulators (Ahmad et al., 2008) have been reported to shown resistance in *S. litura* from different countries viz., China, Pakistan and India.

Metabolic detoxification mechanism with elevated enzyme activities is the main cause of insect pests showing insecticide resistance against a different group of insecticides. Increased level of detoxification enzymes like cytochrome P450 (P450), carboxy/ cholinesterase (CCE), and glutathione S-transferase (GST) is linked to insecticide resistance (Sreelakshmi et al., 2019). There have been reports on poor bioefficacy of the conventional insecticides as well as newer chemicals against *S. litura* in soybean crop and this led to the outbreak of this pest on soybean in various districts in Southern Rajasthan. Therefore, the present study was undertaken to determine the information on resistance in *S. litura* against the new chemistry insecticides and conventional insecticides.

MATERIALS AND METHODS

Susceptible strain (Lab-SS) of *S. litura* obtained from National Bureau of Agriculturally Insect Resources (NBAIR), Bengaluru kept and reared in the laboratory without exposure to insecticides. Insecticide bioassays were performed during 2017 at Agricultural Research Station (ARS), Banswara, Rajasthan to get the data on mortality for different group of insecticides. The field populations of *S. litura* were collected from

soybean crops located in ARS, Banswara (ARSB) and farmers field at Banswara (FFB), Pratapgarh (FFP) districts at Southern Rajasthan, India from 2017-2019 during *Kharif* season. Approximately 100-200 third to fifth instar larvae were collected from soybean crop and reared on natural diet (castor leaves). The culture was maintained in the insect rearing room at ARS, Banswara at a temperature of 25± 1^{0}C, 60± 5% RH and a 14:10 h light: dark cycle. Commercial formulations of pesticides viz., Emamectin benzoate (Proclaim 5% SG; Syngenta India Limited); Chlorantraniliprole (Coragen 18.5% SC; Dupont); Indoxacarb (Avaunt15.8 EC; DuPont); Thiodicarb (Larvin 75% WP; Bayer Crop Science); Cypermethrin (Cymbush 25% EC; Bayer Crop Science); Profenophos Carina 50EC; PI Industries; Triazophos (Josh 40% EC; UPL); Spinetoram (Delegate 11.7% SC; Dow Agrosciences); Novaluron (Rimon 10% EC; Crystal) were used for the bioassay.

The standard leaf dip bioassay (Sayyed et al., 2000; Ahmad et al., 2007b) was used to determine the dose-response for insecticides from different groups in *S. litura*. Various concentrations of insecticides were prepared using distilled water containing 0.1% Triton X-100 and leaf discs (8/5 cm) of castor was dipped into the test insecticides for 15 seconds with gentle agitation. Control discs were treated with water containing 0.1% Triton X-100. The castor leaf discs treated with insecticide or water (control) were dried at room temperature for 1 hr. Treated leaves were then transferred to individual Petri dishes (8/5 cm in diam.) which were lined with moistened with Whatman filter paper. Three third-instar larvae of *S. litura* from F1 generation laboratory cultures were released onto each leaf disc and maintained at 27± 1^{o}C, 60-70% RH with a 14:10 hr light: dark cycle. At least thirty 3rd instar larvae were exposed to each dose for at least six concentrations of each insecticide. Mortalities after 48 or 72 hr of exposure were recorded. The 3rd to 5th instar larvae of *S. litura* were separated and starved for three hr to remove food particles. The crude and the microsomal preparation were prepared according to Mohan and Gujar, 2003; Ramya et al., 2016). The total activities of P450s, ESTs, and GSTs were determined by using crude enzyme preparation. The QuickStart Bradford assay kit of Biorad was used for the estimation of protein content as per Bradford (1976). The cytochrome P450 assay was carried out as per the procedure of Mohan and Gujar (2003) and Kranti (2005). Carboxylesterase activity was determined by the method of Kranti (2005). The GSH-S-transferase enzyme activity in the gut samples of *S. litura* was measured by the method of Mohan and Gujar (2003) and Kranti (2005). The acetylcholinesterase enzyme activity was measured by the method of Kranti (2005). Gel electrophoresis was carried out on Native PAGE with 10% resolving gel for the separation of esterase enzymes of *S.litura* along with the Lab-Susceptible populations as per the procedure of Kranti (2005). Bioassay data of three replicates were pooled and subjected to Abbotts' formula (Abbott, 1925) and analyzed using Indostat Services, Hyderabad.

RESULTS AND DISCUSSION

The results revealed that chlorantraniliprole was the most toxic while the synthetic pyrethroid, cypermethrin and organophosphates (profenophos and triazophos) recorded least toxicity to *S.litura* populations collected from all the three locations (Table 1). The indiscriminate use of these compounds in the region is the cause for the development of resistance. Karuppaiah et al. (2017) revealed that chlorantraniliprole 18.5% SC @ 1-4 ppm (LC_{50}) and emamectin benzoate 5% SG @ 1-3ppm (LC_{50}) was found highly effective. Chlorantraniliprole was the most toxic insecticide against jute hairy caterpillar too (Selvaraj et al., 2015). The field population of *S. litura* showed highest resistance to cypermethrin with resistance ratios (RRs) ranging from 244 to 376. Ruttanaphan et al. (2018) also reported that the field populations from Thailand exhibited resistance to cypermethrin. The resistance to chlorantraniliprole and spinetoram was the lowest in the ARSB and FFP population, respectively. Dhawan et al. (2007), Bhatnagar et al. (2013), Kaur et al. (2007) and Karuppaiah et al. (2017) also reported higher toxicity of chlorantraniliprole and emamectin benzoate. The carbamate, thiodicarb and oxadiazine, indoxacarb evaluated in the present study, had low to moderate levels of resistance and RR values ranged from 10- to 40-fold and 19- to 25-fold, respectively. Tong et al. (2013) recorded low to medium levels of resistance to indoxacarb. Profenophos and triazophos showed RR values from 43 to 172 compared to the Lab-SS strain. Tong et al. (2013) reported a high level of 50 fold resistance in populations from China. In India, Armes et al. (1997) and Kranti et al. (2002) reported resistance to organophosphates. In the case of insect growth regulator, novaluron, populations revealed high to very high-level of resistance and reported RR values ranging from 52- (FFB) to 106-fold (ARSB).

Detoxification enzyme activities, viz., GSH-S-transferase (GST), cytochrome P450 (MFO),

Table 1. Toxicity of insecticides against field populations of *S. litura*

Insecticides	Location	LC50 (PPM)	Fiducial limit (95% CI)		χ^2	Slope (± SE)	RR	Resistance level
			Lower	Upper				
Chlorantraniliprole	FFB	0.78	0.47	1.29	5.49	1.31± 0.14	8.66	Very low
	ARSB	0.36	0.21	0.63	2.93	1.28± 0.14	4.00	Very low
	FFP	1.07	0.64	1.79	0.58	1.06± 0.17	11.88	Low
	Lab-SS	0.09	0.05	0.16	2.10	1.20± 0.15	1.00	-
Indoxacarb	FFB	2.12	1.21	3.73	4.33	0.98± 0.18	21.2	Moderate
	ARSB	1.92	0.98	3.76	6.32	0.78± 0.23	19.2	Low
	FFP	2.57	1.35	4.88	2.40	0.85± 0.21	25.70	Moderate
	Lab-SS	0.10	0.06	0.16	6.40	1.14± 0.16	1.0	-
Thiodicarb	FFB	6.04	2.97	12.27	1.48	0.76± 0.24	35.53	Moderate
	ARSB	6.90	3.61	13.15	3.60	0.81± 0.22	40.58	Moderate
	FFP	1.71	0.77	3.79	6.16	0.89± 0.20	10.05	Low
	Lab-SS	0.17	0.22	0.64	2.07	0.99± 0.18	1.00	-
Emamectin Benzoate	FFB	1.41	0.78	2.56	0.67	1.07± 0.17	17.62	Low
	ARSB	1.09	0.52	2.29	2.79	0.92± 0.19	13.62	Low
	FFP	2.52	1.33	4.76	1.84	0.89± 0.20	31.50	Moderate
	Lab-SS	0.08	0.04	0.17	1.28	0.79± 0.23	1.00	-
Cypermethrin	FFB	15.04	7.24	17.19	5.23	1.47± 0.14	376	Very high
	ARSB	11.16	7.24	17.19	5.23	1.47± 0.12	279	Very high
	FFP	9.76	5.96	14.73	4.53	1.44± 0.12	244	Very high
	Lab-SS	0.04	0.02	0.08	0.24	0.77± 0.11	1.00	-
Spinetoram	FFB	1.06	0.52	2.15	2.81	0.79± 0.10	8.83	Low
	ARSB	1.19	0.64	2.23	4.06	1.01± 0.18	9.91	Low
	FFP	0.71	0.36	1.37	1.36	1.04± 0.17	5.91	Low
	Lab-SS	0.12	0.08	0.18	6.84	1.49± 0.12	1.00	-
Novaluron	FFB	3.64	1.88	7.07	6.47	1.01± 0.18	52	High
	ARSB	7.47	4.24	13.16	3.54	1.01± 0.18	106.71	Very high
	FFP	4.61	2.47	8.60	6.01	0.98± 0.18	65.85	High
	Lab-SS	0.07	0.03	0.15	0.60	0.87± 0.21	1.00	-
Profenophos	FFB	40.52	29.06	56.50	1.88	1.57± 0.11	109.51	Very high
	ARSB	16.04	9.83	26.17	1.42	1.24± 0.14	43.35	Moderate
	FFP	63.93	40.65	100.54	4.27	1.60± 0.11	172.78	Very high
	Lab-SS	0.37	0.22	0.64	2.07	1.00± 0.18	1.00	-
Triazophos	FFB	52.50	31.48	87.56	8.43	1.03± 0.17	78.35	High
	ARSB	41.91	21.46	81.83	10.25	1.10± 0.16	62.55	High
	FFP	85.33	52.00	140.05	3.05	1.27± 0.14	127.35	Very high
	Lab-SS	0.67	0.34	1.31	6.23	0.83± 0.22	1.00	-

Resistance level classified as none (RF= 1), very low (RF = 2–10), low (RF= 11–20), moderate (RF= 21–50), high (RF=51–100) and very high (RF=> 100) (Ahmad et al. 2007)

carboxylesterase (CarE) and acetylcholinesterase (AChE) of *S.litura* are presented in Fig. 1-4. All the enzyme activities were significantly higher in the whole larval extracts and midgut extracts of larvae collected from all three locations when compared to the Lab-SS strain. The whole larval extracts and midgut extracts from the field populations (FFB, ARSB, FFC and FFP) had significantly higher carboxylesterase enzyme activity. Cui et al. (2015) demonstrated that an increased amount of carboxylesterase is a major factor that induces insecticide resistance. Sreelakshmi et al. (2019) also reported that the resistance field populations of *S.litura* exhibited a two to three-fold increase in carboxylesterase (CarE) enzyme activity. Field populations of *S.litura* showed increased GSH-S-transferase enzyme activity in whole larval extracts and midgut extracts which ranged from 5.3 to 13.8 fold and 5.0 to 11.47 fold. In the case of cytochrome P450 the midgut extracts were studied, and o-demethylase activity of FFP and FFC field populations had increased enzyme activity with the 19-26 folds with that of the Lab-SS strain. Similarly, Sreelakshmi et al. (2019) observed the variations in the specific activity profiles of glutathion-S-transferase (GST) in the field populations and the laboratory susceptible strains. Resistant populations exhibited the higher MFO specific

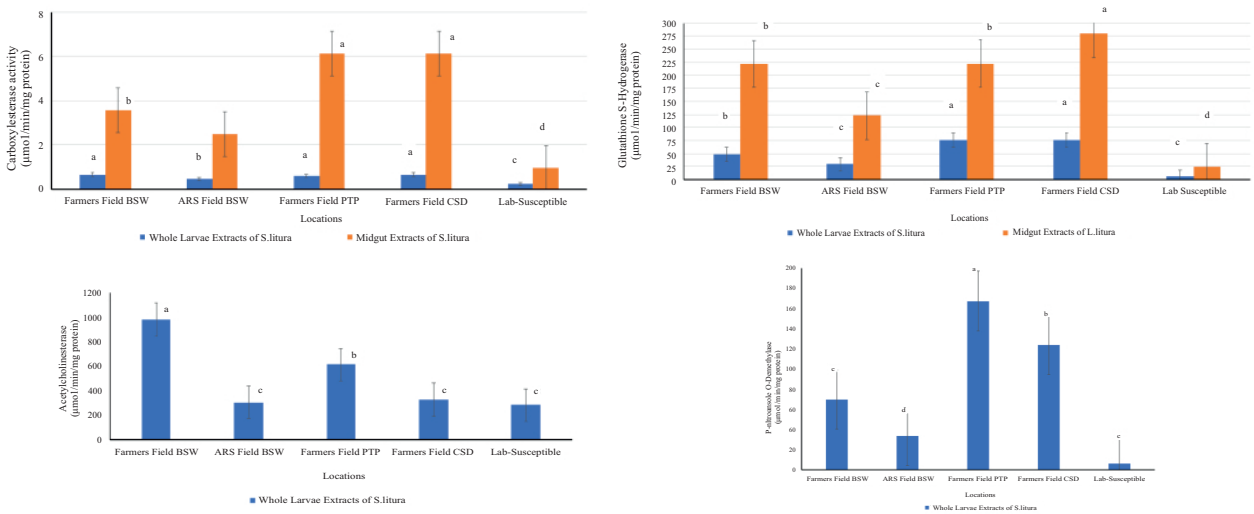

Fig. 1-4. GSH-S-transferase (GST), cytochrome P450 (MFO), carboxylesterase (CarE) and acetylcholinesterase (AChE) activity in *S.litura*

activity when compared to the laboratory susceptible strains in the studies by Huang and Han (2007) and Karuppaiah et al. (2017). This high expression level of MFO specific activity may be the reason for the development of resistance in synthetic pyrethroid. Similarly, MFO activities were found to be higher in the field populations (Sreelakshmi et al., 2019).

Among the different detoxification enzymes studied, the acetylcholinesterase activity was found highest in whole larval extracts of all the field populations of *S. litura*. Sreelakshmi et al. (2019) observed that the field populations of *S.litura* exhibited the 4- to 16- fold increase in specific activity of acetylcholine esterase (AChE). In the present study, a high amount of GST activity was observed in *S. litura* whereas the total enzyme activity is varying with the results of Karuppaiah et al. (2017). Analysis of the carboxyl esterase enzyme in the native PAGE assay revealed that the electrophoretic profiles showed the presence of different band regions (EST 1 to EST 7) (Fig. 5) with esterase activity in the field populations of *S. litura* along with the laboratory susceptible strain. The results demonstrated that the five populations

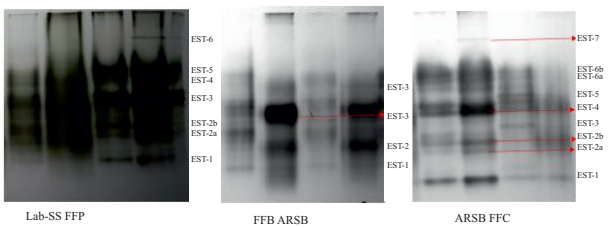

Fig. 5. Carboxyl esterase (CarE) in the native PAGE assay-, electrophoretic profiles showing band regions (EST 1 to EST 7)

and Lab SS of *S. litura* shared some of the similar esterase band patterns. One or two prominent esterase bands were observed in all the field populations except Chottisadri populations whereas, one faint esterase band was present in FFB and FFC populations. Esterase bands 5, 6, and 7 were not visible in the Lab-SS strain of *S. litura*. Ramya et al. (2016) analyzed the electrophoretic profiles of four bands (Est-1, Est-2, Est-3 and Est-4) with CarE activity in the field populations of diamond backmoth and one (Est-2) of these bands in the laboratory susceptible strain.

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EFFECT OF MALE IRRADIATION AND ITS MATING STATUS ON THE REMATING PROPENSITY, INSEMINATION QUALITY AND REPRODUCTIVE BEHAVIOUR OF MALE *SPODOPTERA LITURA* (F.)

Nilza Angmo, Neha Vimal, Madhumita Sengupta and R K Seth*

Applied Entomology and Radiation Biology Unit,
Department of Zoology, University of Delhi, Delhi 110007, India
*Email: rkseth57@gmail.com (corresponding author): ORCID ID- 0000-0001-7873-1362

ABSTRACT

In this study various factors, viz., male irradiation, its mating status and post mating interval were studied on the remating propensity, insemination quality and reproductive behaviour of a noctuid pest *Spodoptera litura* (F.) that may govern the efficacy of radiation mediated inherited sterility (IS) technique proposed to control this pest. The male irradiation (substerilizing 130 Gy and sterilizing 250 Gy) affected the copulation duration, mating success and sperm transfer during mating. Multiple matings of male significantly depleted the sperm in fully sterile male followed by substerile male than control. During remating with virgin female, the reproductive performance of mated irradiated male, was more affected at 24 hr than at 48 hr inter mating interval, which might be due to associated physiological impact on mating behaviour and insemination quality. This study might be crucial in simulation modelling and optimization of successful implementation of IS programme.

Key words: *Spodoptera litura*, male irradiation, 130 Gy, 250Gy, insemination quality, apyrene and eupyrene sperm depletion, F1 sterility technique, multiple mating

For many insects, the optimal reproductive strategies differ between males and females, where males benefit from increased mating frequency (Parker and Birkhead, 2013) whereas the females maximize their egg production by selecting fewer but high quality mates. By acquiring multiple matings, males generally maximize mating success by providing an opportunity for each mating to result in an offspring. The multiple mating in males often has a significant impact on their lifelong reproductive success. According to recent studies, the males can experience significant costs such as sperm depletion and reduced survival due to multiple mating (Wedell et al., 2002). In remating males, successful fertilization may depend on their ability to replenish ejaculatory substances during each mating (Dewsbury 1982). The time interval between first and subsequent matings had a strong effect on the size and protein content of ejaculates (Bissoondath and Wiklund, 1996). The males transfer nutrients to females via ejaculates/ spermatophore, or other nuptial gifts during mating or copulation (Thornhill 1976; Thornhill and Alcock 1983) but the male reproductive potential has been limited by spermatogenesis (Wedell et al., 2002). During mating, the male moths transfer spermatophore containing sperm and accessory gland products to females. The male could affect a recipient's reproductive strategy (Dewsbury, 1982) by affecting female fertility, offspring size or quality, female remating behavior and female longevity (Parker and Simmons, 1989; Boggs, 1990; Wedell, 1996). The nutrients enclosed in spermatophores have been found in the eggs and soma of mated females (Boggs and Gilbert, 1979; Greenfield 1982; Wiklund et al. 1993). According to a meta-analysis of Lepidopterans, increasing mating frequency results in a steady decline in male ejaculate (sperm counts and/or spermatophores mass), which has a negative influence on female fecundity and fertility (Torres-Vila and Jennions, 2005).

The tobacco cutworm *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), is a polyphagous noctuid agricultural and forest pest species. The chemical pesticides are the common method to control the pest population of *S. litura* but it has resulted in an increased resistance towards the insecticides and also affected the human health and environment (Shad et al., 2012). In this context, an eco-friendly bio-rational radio-genetic technique of pest control, commonly known as sterile insect technique (SIT) could be an appropriate option for pest management, which involves mass-rearing, sterilization and release of male moths in the infected area where sterile males must mate with wild females

and prevent them from reproducing by transferring inviable sperm in a sufficient ejaculate including accessory gland fluid (Knipling, 1955; Robinson, 2005). In intrinsically radioresistant Lepidoptera, the use of a high dose of gamma radiation to induce full sterility in male moths may diminish competitiveness and ejaculate quality in irradiated moths. Therefore, an alternate strategy known as F1 or Inherited sterility technique (IS), as a modified SIT was proposed to overcome the limitations of SIT by releasing sub-sterile males with the ability to cause greater sterility in their F1 generation to be used in pest suppression. Gamma radiation often resulted in reduced sperm transfer, which might be drastic if irradiated males mated repeatedly. Male multiple matings (polygamy) could result in aspermia (Hooper, 1989) and possible ejaculate depletion. For example, the sterile males of *Bactrocera cucurbitae* and *B. tryoni* rapidly reached sperm depletion after three consecutive matings (Haynes and Mitchell, 1977; Kuba and Itô, 1993; Radhakrisnan et al., 2009). These findings implied that the combined effect of irradiation and multiple male matings might result in a smaller quantity and/or lower quality of the ejaculate, which could affect the female post-mating behaviour and induction of sterility in F1 generation which might eventually influence the efficiency of this radio-genetic technique. In the present study, an attempt was made to ascertain the effects of male mating history and irradiation on the male reproductive investment in the form of ejaculates transferred during mating, and the insemination quality in the spermatophore (in terms of apyrene and eupyrene sperm transferred) during consecutive matings was examined with an inter-mating interval of 24 and 28 hr (post first mating) in relation to reproductive performance.

MATERIALS AND METHODS

A continuous culture of *S. litura* was maintained in the insectary at the Department of Zoology, University of Delhi, India. The larvae were reared on a semisynthetic diet under ambient environmental conditions with temperature of 27.0± 1°C, 75± 5% RH, and a photoperiod of 12:12 hr (light: dark), also precautionary measures were taken to avoid microbial infection (Seth and Sharma, 2001). Freshly emerged male moths (0-1 day old) were exposed to substerilizing dose of 130 Gy, proposed for the F1 sterility technique against *S. litura* and fully sterilizing dose of 250 Gy (Seth and Sehgal, 1993; Seth and Sharma, 2001) at a dose rate of 0.625-0.359 KGy/ hr in a Co^{60} Gamma Chamber-5000 (GC-5000) located in the radiobiological unit of the Institute of Nuclear Medicine and Allied Sciences (INMAS), Ministry of Defense, New Delhi. For control, the unirradiated male moths were exposed to the same environmental conditions as the irradiated males except the irradiation exposure. To examine the effect of male mating history and irradiation on ejaculate quality, the newly eclosed adults were placed in cohorts of 12-15 pairs in a Perspex-nylon cage (45 x 30 x 30 cm) in the following combinations: [i] unirradiated females (N♀) × unirradiated males (N♂) as control, [ii] N♀ × irradiated males ($T♂_{130}$), irradiated at substerilizing dose,130 Gy and [iii]] N♀ × irradiated males ($S♂_{250}$), irradiated at fully sterilizing dose, 250 Gy. During scotophase, the cages were inspected every 15 min for mating pairs under red light. Individuals observed to mate during that time were collected and held for use in remating trials. Once copulation was terminated, the females were immediately put into a freezer, whereas males were transferred to holding cages where they were fed *ad libitum* until the day they were to be remated.

For remating trials, since no males mated twice in a day; all males mated for the first time (control or irradiated mated male moths) were given the opportunity to remate either after 24 or 48 hr post initial mating with normal females in a cohort of 10-12 pairs/ cage (45x 30x 30 cm). For the purpose of remating, the mated males and virgin females were kept in cages and were inspected every 15 min for mating pairs. Once a copulating pair was detected, the time of initiation and cessation of copulation was recorded to determine the copulation duration. Immediately after mating termination, the females were dissected for the presence of spermatophore in the bursa copulatrix. To examine the number of sperm transferred during mating of moths in a specific regimen, the female moths were dissected in Belar's saline. To count the number of eupyrene sperm bundles, the contents of the spermatophores were spread on a microscope slide for examination by phase contrast microscope. Whereas to determine the number of apyrene sperm (loose sperm) the content of spermatophore was diluted in known volume of Belar's saline and ten 10 ul aliquots were removed from each spermatophore to count the apyrene sperm number by Haemocytometer, and an average reading of these ten aliquots constituted one replicate. The biological replication was performed seven times for examining the ejaculate quality related to sperm number and an average reading in a cohort of 4-5 females in each regimen (control and irradiated) after inter-mating intervals of 24 and 48 hr post initial mating with virgin male constituted one replicate. For ascertaining the

mating and remating success, each replicate comprised of 12-15 pairs of moths in a specific regimen and nine replicates were conducted to compute the results.

Mated females from each regimen were placed in a cage and were provided with an oviposition substrate of castor leaf and 10% honey as a source of food. Each cage was monitored for ovipositional response and the eggs laid were recorded daily. For each experimental regimen, an average reading of oviposition per female from each cage of 4-5 pairs constituted one replicate, and an average reading from 6-7 egg samples from each such cage for testing egg hatchability constituted one replicate. This experiment was repeated seven times for each condition. All statistical analyses were performed using Graph Pad Prism software (version 9.0). One-way ANOVA, followed by Tukey's multiple comparisons was performed on different regimens to analyze the differential effect of multiple matings of irradiated males with different inter-mating interval (24 and 48 hr after first mating) on the male ejaculate quality and other reproductive parameters, in relation to control. The percentage data were arcsine transformed before ANOVA and the data in the table were back transformations.

RESULTS AND DISCUSSION

In this study the effect of multiple matings of males with different inter-mating interval (24 and 48 hr after first mating) was observed on the copulation duration and mating success in irradiated male moths with respect to control (unirradiated male moths). In control (unirradiated moths), the copulation duration with virgin females was found to be affected by the male mating history, the previously mated males took longer time than the virgin males (Table 1). The duration spent in copula during remating (with virgin female) increased to ca. 83 min at post mating interval of 24 hr and ca. 72 min at post mating interval of 48 hr in comparison to first mating in control (untreated male moths) The number of days between consecutive matings significantly affected the copulation duration of second matings. The copulation duration was also influenced by the male irradiation status. The duration of mating of treated males (with virgin untreated females) was increased by ca. 21% in sub-sterile male (130 Gy) and ca. 36% in fully sterile male (250 Gy) in comparison to control (first mating with unirradiated male. The copulatory period of mated irradiated male moth was also increased during remating in both the radiation regimen. The average duration spent in copula during remating was increased by 30-35% at intermating interval of 24 hr and by 15-25% at intermating interval of 48 hr in comparison to first mating of irradiated male moths (130 and 250 Gy) (Table 1). The mating duration of the irradiated male moths (with virgin normal females) was shortened as the post first mating interval was increased. However, the copulation duration was notably increased during the remating of normal as well as irradiated male moths.

The prolonged copulation during remating might be a form of mate guarding (Drummond, 1984) whereby the male ensures that the physiological transition of the female from sexually receptive to a non-receptive state is initiated. An extended copulation might also indicate inadequate ejaculate quantity or difficultly in transferring spermatophore during mating (Sims, 1979). Also, involving the female in a lengthy copulation might effectively limit the females to copulate only once in a day (Vera et al., 2003). The male mating history also influenced the mating success of males, as the remating

Table 1. Influence of irradiation and male mating status on the copulation duration of *S. litura* during its sequential matings

Radiation regimen	Copulation duration (min)			
	First mating (V♂xV♀)	Remating		
		24 hr later (M♂xV♀)	48 hr later (M♂xV♀)	
N♀xN♂	55.33± 2.93 Aa	83.43± 2.92 Ac	71.91± 1.47 Ab	F(2,18)=35.34*
N♀xT♂$_{130}$	67.02± 2.02 Aa	90.71± 4.07 ABc	78.57± 4.04 Ab	F(2,18)=11.38*
N♀xS♂$_{250}$	75.36± 2.14 Ba	98.64± 4.55 Bb	92.86± 3.43 Bb	F(2,18)=12.73*
	F(2,18)=17.61*	F(2,18)=3.79*	F(2,18)=11.36*	

Means ± SE followed by same small letters within a row not significantly different at $p<0.05$; Means± SE followed by same capital letters within a column not significantly different at $p<0.05$ (One way ANOVA followed by Tukey's post hoc test); n=7, N-normal (unirradiated), V-virgin, M- mated, T♂$_{130}$- Male moth irradiated at 130Gy; S♂$_{250}$- Male moth irradiated at 250Gy; *-denotes a significant difference at $p \leq 0.05$

success of mated males was found to be decreased as compared to the first mating of the virgin males. Similar observation was also noticed in *D. melanogaster* (Markow, et al., 1978), *Cnephasia jactatana* (Jimenez and Wang, 2004), *Heliothis virescens* (Klepetka and Gould, 1996) and *Spalangia endius* (King and Fischer, 2009). Gamma radiation also influenced the mating success of male. The fully-sterile males were less successful in mating as compared to the sub-sterile males and unirradiated males (Table 2). The reduced mating success as a function of increasing gamma dose was also reported in *Epiphyas postvittana* (Stringer et al., 2013). The remating success of mated irradiated males was also reduced as compared to first mating of the virgin irradiated males. Further, the remating success of irradiated (130Gy, 250Gy) as well as normal (unirradiated) male moths at 24 hr post first mating interval was markedly reduced as compared to 48h post mating interval (Table 2).

These results suggested that even though the males of *S. litura* were capable of multiple matings, they did not remate with same propensity at all intermating intervals, that might be attributed to the limited amount of the ejaculate resources to be invested over consecutive matings. In this study, the effect of consecutive matings with different inter-mating interval (24 and 48 hr after initial mating) on the sperm number in successive ejaculates of the *S. litura* was also examined in irradiated (sub-sterile and fully sterile) male moths in comparison with control (unirradiated male moths). The amount of eupyrene sperm bundles and apyrene sperm transferred during mating via spermatophores was significantly reduced in the mated male moths in compared to virgin male moths (Table 3, 4) as also reported by Seth et al. (2002b) in *S. litura*. The ability of a mated male to transfer the sperm presumably might get reduced after each mating, indicating that male would fail to replenish their sperm supply fully after mating

Table 2. Influence of irradiation and male mating status on the mating success of *S. litura* during its sequential matings

Radiation regimen	Mating success (%)			
		Remating		
	First mating (V♂xV♀)	24 hr later (M♂xV♀)	48 hr later (M♂xV♀)	
N♀xN♂	72.22± 3.6 Aa	52.78± 3.5 Ab	58.33± 2.9 Ab	F(2,24)=8.95*
N♀xT♂$_{130}$	61.11± 3.7 ABa	41.67± 2.4 Bb	55.56± 3.5 Aab	F(2,24)=9.49*
N♀xS♂$_{250}$	47.22± 2.9 Ba	33.33± 2.6 Bb	41.67± 2.5 Aab	F(2,24)=6.85*
	F(2,24)=13.43*	F(2,24)=11.53*	F(2,24)=8.88*	

Means± SE followed by same small letters within a row not significantly different at p<0.05; Means± SE followed by same capital letters within a column not significantly different at p<0.05 (One way ANOVA followed by Tukey's post hoc test); n=9; An average reading for mating success from a cohort of 12-15 pairs constituted one replicate; N-normal (unirradiated), V-virgin, M- mated, T♂$_{130}$- Male moth irradiated at 130 Gy; S♂$_{250}$- Male moth irradiated at 250 Gy; *-denotes a significant difference at p ≤ 0.05

Table 3. Influence of irradiation and male mating status on the eupyrene sperm transferred by males of *S. litura* during its sequential matings

Radiation regimen	Eupyrene sperm bundles transferred to female via spermatophore			
		Remating		
	First mating (V♂xV♀)	24 hr later (M♂xV♀)	24 hr later (M♂xV♀)	
N♀xN♂	351.7± 8.72 Aa	86.14± 7.636 Ac	199.9± 11.37 Ab	F(2,18)=202*
N♀xT♂$_{130}$	308.4± 10.03 Ba	69.29± 6.69 Ac	158.6± 10.36 Bb	F(2,18)=60.44*
N♀xS♂$_{250}$	159.3± 12.18 Ca	29.86± 4.95 Bc	64.43± 5.47 Cb	F(2,18)=66.43*
	F (2,18)=106.2*	F (2,18)=19.63*	F (2,18)=54.26*	

Means ± SE followed by same small letters within a row ot significantly different at p<0.05; Means± SE followed by same capital letters within a column not significantly different at p<0.05 (One way ANOVA followed by Tukey's post hoc test); n=7; An average reading for eupyrene sperm bundles transferred from a cohort of 4-5 pairs constituted one replicate; N-normal (unirradiated), V-virgin, M- mated, T♂$_{130}$- Male moth irradiated at 130Gy; S♂$_{250}$- Male moth irradiated at 250Gy; *-denotes a significant difference at p ≤ 0.05

Table 4. Influence of irradiation and male mating status on the apyrene sperm transferred by males during its sequential matings.

Radiation regimen	Apyrene sperm x 10^3 transferred to female via spermatophore			
		Remating		
	First mating (V♂xV♀)	24h later (M♂xV♀)	48h later (M♂xV♀)	
N♀xN♂	281.8±7.12 Aa	127.4± 6.40 Ac	231.3± 11.13 Ab	F (2,18)=104.6*
N♀xT♂$_{130}$	227.1± 7.59 Ba	98.21± 9.08 Bc	171.9± 11.79 Bb	F (2,18)=30.82*
N♀xS♂$_{250}$	126.3± 8.37 Ca	49.37± 4.25 Cc	86.29± 4.27 Cb	F (2,18)= 57.52*
	F (2,18)=78.72*	F (2,18)=32.43*	F (2,18)=56.68*	

Means ± SE followed by same small letters within a row not significantly different at $p<0.05$; Means ± SE followed by same capital letters within a column not significantly different at $p<0.05$ (One way ANOVA followed by Tukey's post hoc test); n=7; An average reading for apyrene sperm transferred from a cohort of 4-5 pairs constituted one replicate; N-normal (unirradiated), V-virgin, M- mated, T♂$_{130}$- Male moth irradiated at 130Gy; S♂$_{250}$- Male moth irradiated at 250Gy; *-denotes a significant difference at $p \leq 0.05$

and males need time to mobilize and/or replenish it. The extent of decline in sperm supply during remating was influenced by the interval between consecutive matings. With increase in inter-mating interval there was a significant increase in sperm number observed in irradiated and normal males as noticed in the present study.

Seth et al. (2002b) showed in *S. litura* that the decrease in the number of eupyrene sperm bundles in the duplex on the day after mating might be correlated with the estimates of the number of bundles transferred to the female in the spermatophore during mating. The increased sperm transferred during remating of male after an interval of 48 hr could be due to accumulation of sperm into duplex as Seth et al. (2002b) showed that the rate or extent of sperm descent from the testis to the upper vas deferens (UVD) in adult male *S. litura* did not get affected by mating.

Male moths irradiated with high dose of fully sterilizing gamma radiation (250 Gy) transferred significantly less sperm than male irradiated with substerilizing, 130 Gy. Irradiated males mated to normal females showed similar trend in sperm (eupyrene sperm bundles and apyrene sperm) transfer during remating as observed in unirradiated male (Table 3, 4). Also, the irradiated male moths transferred significantly less amount of sperm during their first as well as subsequent matings when compared with consecutive matings of unirradiated males. Likewise, the increased sperm depletion with subsequent matings of the irradiated male moths was also noticed in *Bactrocera tryoni* (Radhakrishnan et al., 2009). The reduced number of sperms transferred could be due to effect of irradiation on sperm production and/ or sperm descent from the testes (Seth et al., 2002a). The extent of decline in sperm transfer was found to be influenced by irradiation dose (130, 250 Gy) as well as the interval between the consecutive mating (24, 48 hr post initial mating) in *S. litura.*

In Lepidoptera, the newly emerged adult males have sperm and late spermatid stages (Holt and North 1970; Ashrafi and Roppel, 1973). The radiosensitivity of germ cells has been classified, in decreasing order, as follows: spermatogonia cells, spermatocytes, spermatids and the most resistance stage is the mature sperm (Hodges, 1980). Therefore, if newly emerged adult males are irradiated, their spermatids would be more affected and would suffer greater damage than the mature sperm which might be one of the reasons in the decline of sperm transfer observed during subsequent mating of irradiated moths and the effect was more pronounced at high dose of gamma radiation (250 Gy). Further, the effect of male mating history on the oviposition behavior and egg viability was investigated in *S. litura* by pairing normal females with virgin and mated males at different intermating interval of 24 and 48 hr vis-à-vis first mating. The oviposition (eggs laid/ mated female) and egg fertility were reduced in case of females crossed with mated than virgin male. These reproductive responses (ovipositional response and egg fertility) in *S. litura* were more adversely affected due to remating of the mated male at 24 than 48 hr intermating interval (Table 5, 6). Similar influence of the male mating history on the oviposition of females was observed in *Colias eurytheme* (Rutowski et al., 1987), *Ostrinia nubilalis* (Royer and McNeil, 1993) and *Helicoverpa armigera* (Hou and Sheng, 1999), as well as the egg viability of *H. virescens* (Henneberry and Clayton, 1985), *O. nubilalis* (Royer and McNeil, 1993)

Table 5. Influence of irradiation and male mating status on the oviposition of females of *S. litura* crossed with these males during its sequential matings

Radiation regimen	Oviposition per female			
	Remating			
	First mating (V♂xV♀)	24 hr later (M♂xV♀)	24 hr later (M♂xV♀)	
N♀xN♂	1685± 31.13 Aa	933.1± 25.96 Ac	1489± 24.42 Ab	F(2,18)=203.9*
N♀xT♂$_{130}$	1118± 27.64 Ba	378.2± 34.24 Bc	871.3± 30.29 Bb	F(2,18)=148.8*
N♀xS♂$_{250}$	741± 28.04 Ca	181± 26.46 Cc	389± 18.28 Cb	F(2,18)=132.2*
	F(2,18)=268.9*	F(2,18)=179.2*	F(2,18)=493.6*	

Means ± SE followed by same small letters within a row are not significantly different at p<0.05; Means ± SE followed by same capital letters within a column not significantly different at p<0.05 (One way ANOVA followed by Tukey's post hoc test); n=7; An average reading for oviposition (eggs/mated female) from a cohort of 4-5 pairs constituted one replicate; N-normal(unirradiated), V-virgin, M- mated, T♂$_{130-}$ Male moth irradiated at 130Gy; S♂$_{250-}$ Male moth irradiated at 250Gy; *-denotes a significant difference at p ≤ 0.05

Table 6. Influence of irradiation and male mating status on the egg fertility in females crossed with these males during its sequential matings

Radiation regimen	Egg fertility (%)			
	Remating			
	First mating (V♂xV♀)	24 hr later (M♂xV♀)	24 hr later (M♂xV♀)	
N♀xN♂	85.48± 3.55 Aa	67.03± 2.89 Ab	76.41± 3.94 Aab	F(2,18)=6.99*
N♀xT♂$_{130}$	45.49± 2.03 Ba	11.32± 2.5 Ac	29.47± 1.03 Bb	F(2,18)=85.71*
N♀xS♂$_{250}$	0Ca	0Ca	0Ca	F(2,18)=1
	F(2,18)=328.1*	F(2,18)=264.5*	F(2,18)=268.6*	

Means ± SE followed by same small letters within a row not significantly different at p<0.05 level; Means ± SE followed by same capital letters within a column not significantly different at p<0.05 level (One way ANOVA followed by Tukey's post hoc test); n=7; An average reading for egg fertility from a cohort of 4-5 pairs constituted one replicate; N-normal(unirradiated), V-virgin, M- mated, T♂$_{130-}$ Male moth irradiated at 130 Gy; S♂$_{250-}$ Male moth irradiated at 250 Gy; *-denotes a significant difference at p ≤ 0.05.

and *Epiphyas postvittana* (Foster and Ayers, 1996). In these examples, both oviposition and egg viability of female mated to previously mated males got decreased.

The irradiation of male moths had a gradational effect on the ovipositional response and egg fertility with drastic impact at 250 Gy (fully sterilizing dose) than 130 Gy (partially sterilizing dose) (Table 5,6). The oviposition and egg fertility of female copulated with mated irradiated male moths was significantly reduced than those mated with virgin irradiated male moths. The most apparent explanation for the effect of male mating history on female ovipositional response might be the reduced ejaculate quality in form of sperms transferred to female during insemination. The present findings showed that females might alter their investment on egg production based on male insemination quality. The females who receive low sperm count are more likely to have lower reproductive output, demonstrating cryptic female choice through differential allocation based on the quality of her mate and/ or the time and energy they devote to attract another mate (Wedell, 1996; Wedell and Karlsson, 2003). Even though the male reproductive success could be related to the number of females they would mate, the multiple matings assessed in *S. litura* in this study indicated a lower sperm count with reduced fertilization ability. Similar observation was also reported in *S. littoralis* (Sadek, 2001). Further, as per the present study, the irradiated males seemed to exert its sterilizing effect in its first mating through dominant lethal mutations (DLM), although further added impact on its reproductive performance was apparent in its sequential remating, presumably due to associated physiological adversity in sperm dynamics.

In this study, the findings reflected that male mating history (virgin or mated) and irradiation affected the ejaculate quality and sperm transfer that would be vital in the induction of sterility and mating competitiveness of the irradiated moths to be employed in IS programme.

The results also indicated that the mating could be costly to male as copulation depleted the male access to ejaculate constituents as evidenced by the fact that copulation durations were longer and ejaculates smaller in matings involving the mated males. Hence, this study might help in better understanding of SIT/ IS technique for *S. litura* and optimize its operational logistics.

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AUTHOR CONTRIBUTION STATEMENT

RKS conceived and designed research. NA conducted experiments. NV and MS assisted in the conducting experiments and validation of data. RKS and NA analyzed the data and involved in writing, editing and reviewing the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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ASSESSING THE RADIATION HORMESIS ON THE REPRODUCTIVE BEHAVIOUR OF MALE *SPODOPTERA LITURA* (F.) TREATED WITH LOW DOSE IONIZING RADIATION IN THE PRE-IMAGINAL STAGES

Neha Vimal, Madhumita Sengupta, Nilza Angmo and R K Seth*

Applied Entomology and Radiation Biology Unit, Department of Zoology,
University of Delhi, Delhi 110007, India
*Email: rkseth57@gmail.com (corresponding author): ORCID ID- 0000-0001-7873-1362

ABSTRACT

The quality of the mass reared insects plays a crucial role in effective operation of radiation mediated inherited sterility (IS) programme, proposed to suppress the lepidopteran pest *Spodoptera litura* (F.). In this context, the low dose of ionizing radiation (LDIR) was assessed to have any stimulatory effect on the reproductive behaviour of male moths treated as 0-1 day old egg, third instar larva (L3) or 2-3 days old pupa. Radiation hormesis was observed in the mating success of males treated at 0.75 Gy and 1 Gy in both egg and L3 stages. Invitro sperm activation assay indicated more sperm activity during peak plateau phase in moths derived from treated egg (0.75Gy, 1 Gy) and treated L3 (1 Gy). These findings implicit beneficial effect on sperm activity and mating success of male *S. litura,* which might further improve mating competitiveness of male moths to be used in IS technique.

Key words: *Spodoptera litura*, male hormesis, low dose ionizing radiation (LDIR), sterile insect technique (SIT), F1 sterility, preimaginal stage, reproductive behaviour

Hormesis is characterized by the production of biological response in an organism or cell in response to low dose of physical or biologic agents whereas higher dose of the same agent has an inhibitory effect (Calabrese and Baldmin, 2003; Calabrese, 2008). This nonlinear/biphasic dose response can be seen from bacteria to vertebrates by various different stressors. It has now been observed in a wide range of organisms in response to many stressors like alkylating agents, thermal and oxidative stresses, ionizing radiation, chemical stressors, heavy metals etc. (Berry and López-Martínez, 2020). At low dose, a wide range of biological effects are induced which may modulate the physiological response (Luckey, 1999; Calabrese and Blain, 2005; Lefcort et al., 2008). *Spodoptera litura* (F.) is a polyphagous owlet moth that wreaks havoc on a variety of plants all around the world (Bragard et al., 2019). Sterile insect technique (SIT) is a unique radiogenetic strategy for controlling lepidopteran populations in an afflicted field and has been used to control these moths. The use of a high dose of ionizing radiation to produce sterile insects reduces the competitiveness of the irradiated moths (Seth and Reynold, 1993; Bakri et al., 2005). As a result, the F1 (inherited) sterility (IS) technique has been proposed to overcome this limitation of SIT, in which moths are exposed to a much lower dose of ionizing radiation and sub-sterile male insects are produced, and the progeny of these moths would be fully sterile.

Despite being an environment friendly and non-polluting method, it has some constraints like exposing an insect to sterilizing dose of gamma radiation can decrease the mating ability and reduce lifespan in comparison to wild or non-irradiated moths, and these factors can affect the fitness of insect pests (Shelly et al., 1994; Lance et al. 2000; Seth and Sharma, 2001). Lepidopteran insects have dichotomous spermatogenesis i.e. they have two concomitant types of spermatozoa which differ in their structure, DNA content and their differentiation. One form of sperm is nucleated (eupyrene) that can fertilize the egg (e.g., Baccerti, 1991). The other one, sometimes known as parasperm (Jamieson, 1987), is anucleated (apyrene) sperm that cannot fertilize the eggs. Apyrene sperms, on the other hand, play a role in sperm competition and proper functioning of eupyrene sperm (Silberglied et al., 1984; Gage and Cook, 1995). Sperm dynamics is considered as a crucial factor for insect's viability and mating competitiveness (Seth et al., 2016). Hence, it is important to maintain a reasonable level of the life span, insemination quality and mating competitiveness of the irradiated pest moths that would be employed in F1 sterility technique for pest suppression. Thus, the

purpose of this study was to investigate the stimulatory effect of low dose of gamma radiation on various male reproductive parameters, viz., mating success, mating frequency, sperm behaviour and fertility of *S. litura* treated with probable hormetic doses in early preimaginal stages, which might improve the male mating performance in radiogenetic IS technique.

MATERIALS AND METHODS

The adult *S. litura* of both sexes were collected from agricultural fields of Delhi, India and culture was maintained in an inhouse experimental facility at 27± 2ºC with 75± 5% RH, 12 hr:12hr (light: darkness) regimen. The adult moths were kept in humidified Perspex–nylon net cages in a size of 20x20x20cm for mating and provided with ad libitum supply of 10% honey solution. The adult moths were caged in 4 pairs and post mating and caster leave was put as ovipositional trap. The eggs were collected from the cage in a plastic container (10 cm diameter x 12 cm height) and freshly hatched larvae were reared on semi-synthetic diet until pupation, and the rearing cycle continued (Seth and Sharma, 2001). For the experiment, eggs (0-1day old), third instar larvae (L3) and pupae (2-3 days old) were exposed in different regimens to a range of 0.25-1.25 Gy low dose ionizing radiation (LDIR) in a Co^{60} Teletherapy unit (Bhabhatron-II) situated at Institute of Nuclear Medicine and Allied Sciences (INMAS) of Defense Research and Development Organization (DRDO), Delhi. In this experiment the non-irradiated insects in a particular preimaginal stage of the respective regimen were used as control. The dose rate of gamma radiation ranged from 0.746 Gy-1.099 Gy min^{-1} during the experimentation period.

The adult male exposed to LDIR at preimaginal stages were used for the sperm activation assay. An invitro sperm bioassay was performed by the procedure described by Seth et al. 2016 to assess the effect of LDIR on sperm motility. Sperm and activator were produced as described below for the in-vitro sperm assay. Duplex (ductus ejaculatorius duplex) was dissected out for sperms and secretions from the prostatic part (ductus ejaculatorius simplex) was used for activator preparation. 0.3 M HEPES-KOH buffer (at pH 7.0) containing 20 mg/mL bovine serum albumin (BSA) was used for sperm and 0.03 M ammonium bicarbonate-acetic acid buffer (at pH 7.0) was used for the activator (prostatic part secretions). The prostatic part was kept in 40 µL of ammonium bicarbonate-acetic acid buffer at pH 7.0. The secretion oozed out in this solution was centrifuged at 4°C for 10 min at 6000 rpm. Sperm activation was performed by mixing sperm from duplex and activator from prostatic part in 1:1 ratio at 27± 1°C to observe the temporal profile of sperm activity under compound microscope (at 400x). For this experiment, each replicate constituted a mean of 5 readings from each aliquot of sperm+ activator and the experiment repeated 10 times for each regimen.

Immediately after termination of mating (LDIR treated male x normal female), the spermatophore was isolated and the sperms were extracted. For sperm count, total number of apyrene sperm (loose sperm) and eupyrene sperm (sperm bundles) were examined under the phase contrast microscope. The content of the spermatophore was spread on a microscope slide for examination of eupyrene sperm bundles, whereas for apyrene sperm count, the content of the spermatophore was diluted with Belar's saline and 10 µl aliquots were examined in a haemocytometer. An average reading of these ten sample aliquots constituted one replicate. For this experiment, 6 replicates were taken for each regimen. The mating status of female was determined by dissecting the female immediately after death and examining the presence of spermatophore in bursa copulatrix. The mating success was determined by assessing the number of females mated out of total females under observation. The mating success was studied in ten replicates, each replicate comprising of 12-15 pairs in a cage (60x 60x 60 cm). The mating frequency was determined by observing the average number of spermatophores in the bursa copulatrix of experimental females that were mated (Seth and Reynolds, 1993; Makee and Saour, 2001).

After the emergence of the adult moths, the irradiated males were paired with virgin non-irradiated female moths and transferred to the mating cages. For control, the non-irradiated virgin males and females were paired. Each mating cage contained 4-5 pairs of insects and were provided with ab libitum supply of food (10% honey solution). Each mating cage was monitored, the egg masses were collected and counted daily during the oviposition period. For each experimental regimen, an average fertility of 10 batches of egg masses (comprising of 70-100 eggs per batch) constituted one replicate. This experiment was repeated 10 times for each regimen. One way ANOVA was performed to determine the evident effects of possible hormetic gamma doses on adult parameters (mating success, mating frequency, egg viability) followed by Tukey's test to confirm the statistically significant difference among the groups at $p<0.05$ level. All the

bio-statistics was carried out using GraphPad prism software program, version 9.3.1 (San Diego, USA).

RESULTS AND DISCUSSION

Dose response plays a central role in biological system as it helps in understanding the biology of a cell or organism by toxicologists, biologists, and pharmacologists (Calabrese, 2004). The term hormesis was given by toxicologists, Chester Southam and John Ehrlich in 1943. Hormesis is generally described as dose response phenomenon in which low dose of stressor elicits a response while high dose of the same stressor can inhibit the response. These stressors can be physical, chemical, radiation, heat stress, oxygen, overcrowding etc. (Sinclair and Howitz, 2005). Biphasic dose response is a fundamental feature of hormesis (Calabrese, 2005; Calabrese and Baldwin, 2001). A significant correlation between hormetic effects and the age of exposure in an organism has also been found in many experiments. It has been observed these hormetic exposures when applied at early life stages show long lasting and better results than at later stages of their lifetime (Le Bourg, 2005; López-Martinez and Hahn, 2014; López-Martinez, 2014; López-Martinez, 2016a; López-Martinez, 2016b; Visser, 2018).

Therefore, in the present study, the effect of low range ionizing doses (0.25 – 1.25 Gy) was evaluated in various preimaginal (egg, L3 and pupa) stages of *S.litura* in order to ascertain the hormetic effect of LDIR in reproductive behaviour of male moths, derived from these treated ontogenic stages. The male reproductive behaviour was assessed in terms of mating frequency, mating success and fertility. Interestingly, the mating success was apparently increased at 0.75 Gy and 1 Gy LDIR in egg stage ($F_{(5,54)}$ =10.95, p<0.001); 0.75 and 1.0 Gy LDIR in larval stage ($F_{(5,54)}$ =5.508, p=0.0002). In pupal LDIR treatment, the mating success of adult moths however did not show any significant difference at 1 Gy with respect to control (0 Gy), indicating that LDIR at 1 Gy didn't induce stressful impact on mating behaviour unlike its other range of doses tested (0.25-0.75 Gy) wherein the mating success was reduced with respect to control. The effect of LDIR investigated on mating frequency, oviposition and egg fertility could not exhibit any hormetic effect as compared to control (0 Gy) (Table 1 a,b,c). Thus, as per the present findings, the radiation hormesis was apparently observed on the mating performance of *S.litura* derived from LDIR treated egg and L3 stages. Similar result was observed by Lalouette et al. (2016) in *Spodoptera littoralis* when low dose of deltamethrin (~1/10 of the LD_{50}) resulted in improved mating success.

Further, the sperm activity of *S.litura* was observed at the probable hormetic doses, viz., 0.75, 1 Gy (egg), 1 Gy (L3) and 1 Gy (pupa), in view of the hormetic effects exhibited in terms of longevity and survivorship at these doses in our initial studies (Seth et al. unpublished). The sperm activity was ascertained as the proportion of sperms being active in *in-vitro* sperm activation assay during 5-135 min period. LDIR treatment at 1 Gy (egg), 1 Gy (larva) and 1 Gy (pupa) showed more sperm activity after 5 min of mixing sperm and activator in comparison to the control. After 15 min, the male moths derived from egg (0.75 Gy), egg (1 Gy) and L3 (1 Gy) were found to elicit more sperm activity with respect to non-irradiated moths. There were evidently more active sperms in the male moths derived from egg (0.75 Gy), egg (1 Gy) and L3 (1 Gy) during peak plateau phase of sperm activity (from 30 to 90 min after mixing sperm and activator) as compared to control (Table 2a). The effect of LDIR was also investigated on the apyrene and eupyrene sperm count but it did not exhibit statistically significant difference at any of the probable hormetic doses. Although, an increasing trend could be seen in moth sperm from 1 Gy treated egg stage but it was not statistically significant (Apyrene sperm $F_{(4,25)}$ =0.39, p=0.81) (Eupyrene sperm $F_{(4,25)}$ =0.48, p=0.75) (Table 2b).

These results suggested a positive role of low dose of ionizing radiation on *S. litura* when exposed in early preimaginal stages, which could be used in IS program because rearing of quality insects is a vital feature in this program. These results suggested that LDIR might increase the mating competitiveness of radiosterilized male moths which might be able to compete better with the wild male in comparison to the ones which were only irradiated with sub-sterilizing gamma irradiation. Thus, these probable hormetic doses might be used to enhance the efficiency and effectiveness of IS programme for this lepidopteran pest suppression.

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Table 1a. Effect of low dose ionizing radiation (LDIR) on reproductive parameters of male *S. litura* treated in egg stage

Reproductive parameters	Low dose ionizing radiation (LDIR) doses						
	Control (0Gy)	0.25 Gy	0.5 Gy	0.75 Gy	1 Gy	1.25 Gy	F value
Mating Success	83.130± 3.08a	83.02± 4.08a	77.43± 6.23a	87.98± 4.08ab	91.84± 2.5b	82.98± 4.08a	10.95 (5,54)*
Mating Frequency	1.6± 0.13a	1.6± 0.08a	1.6± 0.10a	1.5± 0.03a	1.8± 0.14a	1.5± 0.06a	2.55(5, 54)
Oviposition	1806± 56.52a	1694± 57.78a	1696± 52.84a	1771± 60.85a	1781± 41.36a	1623± 73.10a	1.64 (5,54)
Fertility (%)	82.6± 3.76a	80.3± 3.73a	78.8± 4.58a	78.7± 3.25a	80.2± 2.55a	78.5± 3.90a	4.09(5, 54)

Means followed by same letters within a row are not significantly different at $p<0.05$. One-way ANOVA followed by Tukey's multiple comparisons ($p<0.05$) was performed to test the significance among different regimens for a specific parameter. % data was arcsine transformed before ANOVA. n=10; 10-15 pairs constituted one replicate for mating success and average reading from a cohort of 4-5 pairs constituted one replicate for mating frequency, oviposition and fertility. Asterisks (*) signifies statistical significance at $p\leq0.05$

Table 1b. Effect of low dose ionizing radiation on adult parameters of *S. litura* treated as thirds instar larvae (L3)

Reproductive parameters	Low dose ionizing radiation (LDIR) doses						
	Control	0.25 Gy	0.5 Gy	0.75 Gy	1 Gy	1.25 Gy	F value
Mating Success	84.72± 2.88a	84.76± 2.59a	82.22± 5.52a	87.16± 3.52ab	92.99± 2.50b	82.95± 4.08a	5.51(5,54)*
Mating Frequency	1.8± 0.12a	1.7± 0.06a	1.6± 0.08a	1.8± 0.19a	1.8± 0.15a	1.7± 40.11a	2.12(5, 54)
Oviposition	1734± 49.81a	1625± 58.72a	1628± 42.7a	1590± 57.73a	1624± 48.8a	1583± 63.70a	1.49(5,54)
Fertility (%)	81.9± 2.49a	79.2± 2.62a	76.4± 2.63a	78.5± 2.63a	80.5± 1.70a	79± 2.55a	2.40(5, 54)

Means followed by same letters within a row not significantly different at $p<0.05$. One-way ANOVA followed by Tukey's multiple comparisons ($p<0.05$) was performed to test the significance among different regimens for a specific parameter. % data was arcsine transformed before ANOVA. n=10; 10-15 pairs constituted one replicate for mating success and average reading from a cohort of 4-5 pairs constituted one replicate for mating frequency, oviposition and fertility. Asterisks (*) signifies the statistical significance at $p\leq0.05$

Table 1c. Effect of low dose ionizing radiation on adult parameters of *S.litura* treated in pupal stage

Reproductive parameters	Low dose ionizing radiation (LDIR) doses						
	Control	0.25 Gy	0.5 Gy	0.75 Gy	1 Gy	1.25 Gy	F value
Mating Success	85.9± 2.08b	83.5± 3.08a	78.3± 6.24a	79.9± 4.08a	91.6± 2.5b	77.9± 4.08a	8.94(5,54)*
Mating Frequency	1.8± 0.08a	1.7± 0.07a	1.7± 0.12a	1.8± 0.15a	1.8± 0.18a	1.9± 0.13a	0.84 (5, 54)
Oviposition	1651± 61.95a	1541± 66.98a	1569± 38.84a	1586± 55.55a	1590± 48.19a	1509± 60.37a	0.74 (5, 54)
Fertility (%)	84.1± 2.07a	81.04± 3.80a	78.9± 2.94a	78.8± 2.69a	81.4± 2.04a	78.1± 2.89a	5.82 (5, 54)

Means followed by same letters within a row are not significantly different at $p<0.05$. One-way ANOVA followed by Tukey's multiple comparisons ($p<0.05$) was performed to test the significance among different regimens for a specific parameter. % data was arcsine transformed before ANOVA. n=10; 10-15 pairs constituted one replicate for mating success and average reading from a cohort of 4-5 pairs constituted one replicate for mating frequency, oviposition and fertility. Asterisks (*) signifies statistical significance at $p\leq0.05$

Table 2a. Effect of low dose ionizing radiation on sperm activation in male *S.litura* treated in probable hormetic doses (egg, larva, pupa- invitro assay)

LDIR exposure to preimaginal stage	% sperm active in moths derived from LDIR treated pre-imaginal stages (Time period after mixing sperm with activator)							
	5	15	30	45	60	90	105	135
Control (0Gy)	20.88± 1.6a	70.38± 1.8bc	89.69± 2.7ab	87.43± 2.8a	82.39± 2.8a	71.36± 3.2a	54.84± 3.1a	26.4± 1.1a
0.75Gy Egg	19.28± 1.5a	64.63± 2.4ab	86.57± 1.9a	89.21± 2.2a	88.78± 2.3ab	81.1± 3.7ab	53.41± 4.2a	23.26± 2.01a
1Gy Egg	24.62± 1.6ab	75.62± 1.8c	96.41± 1.5b	92.64± 1.9a	92.36± 2.01b	85.32± 2.1b	60.86± 4.5a	26.91± 2.6a
1Gy Larva	26.96± 1.2b	73.64± 2.1bc	93.92± 1.9ab	91.37± 2.8a	93.32± 2.5b	84.26± 3.2b	57.39± 3.2a	26.04± 1.6a
1Gy Pupa	23.03± 1.6ab	62.39± 2.2a	90.36± 1.8ab	90.75± 3.2a	87.83± 2.6a	73.13± 3.6a	55.28± 1.4a	25.37± 1.8a
F Value	4.03(4,45)*	7.69(4,45)*	2.67(4,45)*	0.86(4,45)	2.59(4,45)*	3.98(4,45)*	0.68(4,45)	0.56(4,45)

Means followed by same letters within a row not significantly different at $p<0.05$. One-way ANOVA followed by Tukey's multiple comparisons ($p<0.05$) was performed to test the significance among different regimens for a specific parameter. % data was arcsine transformed before ANOVA. n=10; sperms were derived from duplex and activator secretion from prostatic part of male reproductive tract. Asterisks (*) signifies the statistical significance at $p\leq0.05$

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AUTHOR CONTRIBUTION STATEMENT

RKS conceived and designed research. NV conducted experiments. NV, MS and NA assisted in the conducting experiments and validation of data. RKS, NV analyzed the data and involved in writing, editing and reviewing the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Table 2b. Effect of low dose ionizing radiation (LDIR) on dichotomous sperm count of male *S. litura* treated with probable hormetic doses (egg, larva and pupa)

Type of sperm	Male moths derived from LDIR treated in preimaginal stages					
	Control (0Gy)	0.75 Gy- Egg	1 Gy- Egg	1 Gy- Larva	1 Gy- Pupa	F value
Apyrene sperm $x10^3$	279.7± 5.8a	276.9± 8.9a	287.9 ± 8.4a	275.5± 7.3a	276.6 ± 8.9a	0.39 (4,25)
Eupyrene sperm bundles	347.5± 8.9a	338.5± 15.9a	358.167± 9.9a	350.67± 8.5a	340.167± 12.6a	0.48 (4,25)

Means followed by same letters within a row are not significantly different at $p<0.05$ level. One-way ANOVA followed by Tukey's multiple comparisons ($p<0.05$) was performed to test the significance among different regimens for a specific parameter. n=6, Asterisks (*) signifies the statistical significance at $p\leq0.05$

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EFFECT OF IONIZING RADIATION ON PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDE *(PBAN)* GENE EXPRESSION AND ITS PHOTOSENSITIVE RHYTHM IN FEMALE *SPODOPTERA LITURA* (F.)

Madhumita Sengupta, Nilza Angmo, Neha Vimal and R K Seth*

Applied Entomology and Radiation Biology Unit, Department of Zoology,
University of Delhi, Delhi 110007, India
*Email: rkseth57@gmail.com (corresponding author): ORCID ID- 0000-0001-7873-1362

ABSTRACT

In this study, the effect of ionizing radiation was studied on the pheromone biosynthesis activating neuropeptide *(PBAN)* gene expression in female moths of a noctuid pest *Spodoptera litura* (F.), that would trigger pheromone production and its release needed for calling behaviour. The *PBAN* gene expression in the radiosterilized female moths (at 130 Gy) showed a decline with respect to control (unirradiated moths), and *PBAN* expression was drastically reduced at higher dose, 200 Gy. The photosensitivity and diel rhythm of *PBAN* gene expression, indicating higher expression during peak hours of scotophase and lowest expression during photophase was maintained at 130Gy, which deemed this gamma dose a suitable sterilizing dose for female moths which seemed to retain reproductive competence. This study might support the radiosterilized female moths for their combined release with substerilized males, using 130Gy to effectively operate the F1 sterility technique proposed to control *S. litura*

Key words: *Spodoptera litura*, F1 sterility, radiogenetic pest control, PBAN, gene expression, photosensitivity, diel rhythm, parabiological control, pheromone reduction, calling behaviour

Spodoptera litura (F.) (Lepidoptera: Noctuidae), the common cutworm, is a serious polyphagous pest economically devastating a variety of crops all across India (Moussa et al., 1960). Continuous application of traditional pest control method of using chemical pesticides has led to the development of resistance in this insect while also harming the environment in the process (Armes et al., 1997). The sterile insect technique (SIT), a biorational pest control method, though successful in dipteran pest control, has been less efficient in eradicating lepidopteran pests due to their high radioresistance as lepidopterans require large doses of radiation for 100% sterilization, leading to somatic damage and reduced competitiveness in the irradiated insect (Anisimov et al., 1989). Hence, a modified form of SIT (F1 sterility technique) has been proposed for the control of lepidopteran pests including *S.litura* (Bughio, 1988; Carpenter et al., 1983; 1986, Carpenter, 1987; North, 1975; North and Holt, 1969; Seth and Sharma, 2001; Seth et al., 2016a,b.), wherein the lower (partial sterilizing) dose of gamma radiation is used that would lead to sub-sterile male and eventually sterile F1 progeny in the field. Conventional SIT and F1 sterility technique make use of irradiated male moths and their release in the pest infested field (North, 1975). The use of treated females in the SIT/IS programmes has also been proposed for simultaneous release in various studies and simulation models (Hight et al., 2005; White et al., 1976; Vreysen, 2006). The inclusion of irradiated females along with males might be beneficial in ultimately eradicating the laborious process of sex-based pupal or adult segregation (Braganca et al., 1998). Simultaneous male and female release might also be effective in overwhelming the pest population already present in the field as both treated sexes would compete with wild males and females at the same time (Marec and Vreysen, 2019).

Due to radiogenetic damage caused by gamma radiation in the insects, their reproductive fitness might be affected, that would ultimately affect their performance in the field by hindering their competitiveness against the wild population. Therefore, analysis of effect of radiation on reproductive fitness of the irradiated females is necessary to understand their mating competitiveness with the wild population and contribution in pest control. The premating efficacy of moths is an important indicator of their reproductive fitness concerned with mating ability and mating success. The sexual communication between sexes in lepidopteran species is mediated mainly by sex pheromones, which are volatile compounds used by

the female to attract potential mates from a distance (CSIRO, 1982). Sex pheromones play an important role in the elicitation of mating behaviour in moths and are, therefore, crucial for successful mating. Therefore, understanding the mechanisms that underlie sex pheromone production and the factors influencing it carries a great relevance.

Mating in moths usually occurs during a discrete period of the photophase/scotophase cycle, and in most cases is nocturnal (Jurenka, 2017). Sex pheromone biosynthesis in moths is affected by a variety of exogenous and endogenous factors such as temperature, photoperiod, host plants, age and mating, radiation as well as by endocrine and neuroendocrine factors (Eliyahu et al., 2003; Babilis, 1992; Raina et al., 1991; Xu et al., 1995, Flint, 1983). Raina and Klun (1984) reported that pheromone production in female *Helicoverpa* (then *Heliothis*) *zea* moths was controlled by a cerebral neuropeptide, which was termed pheromone biosynthesis activating neuropeptide (PBAN). PBAN was found to be a 33-amino acid, C-terminally amidated neuropeptide and the peptide was termed Hez-PBAN (nomenclature according to Raina and Ga¨de). It is generally presumed that pheromone production in many lepidopteran species is controlled by PBAN, as is the case in *S. litura* (Abernathy et al., 1995; Choi et al., 2009 and 2012; Chang, 2014; Fabrias et al., 1994; Matsumoto et al., 1995; Lu et al., 2015). In this insect, PBAN released by the brain+ suboesophageal ganglia (brain+ SOG complex) is transported through the haemolymph and targets the PBAN-receptor present on the pheromone glands to initiate secretion of pheromones in the same (Masler et al., 1994; Rafaeli, 2009; Jurenka, 2017). PBAN is produced by the *PBAN* gene which is photosensitive and shows a specific circadian rhythm in production and release depending on light (Zavodska et al., 2009; Iglesias et al., 1999, 2002).

In this study, the light dependent circadian rhythmicity of *PBAN* gene expression was analysed in the sterilized female moth, *S. litura* in view of the exclusively nocturnal mating behaviour exhibited by these moths. The female moths in this study were irradiated with 130 Gy identified as an appropriate complete sterilizing dose, which was also a partial sterilizing gamma dose proposed for male moths for use in F1 sterility technique) (Seth and Sharma, 2001; Seth et al., 2016 a,b; Seth et al., unpublished). Further, a higher dose of 200 Gy was also evaluated in female moths, to assess the gradational response of radiation. These irradiated female moths were studied for relative *PBAN* gene expression at scotophase and photophase to compare the effect of radiation on rhythmic expression of the gene.

MATERIALS AND METHODS

The culture of *S. litura* was maintained at 27±1ºC, 70± 5% RH and 12 hr light : 12 hr dark as photoperiodic regimen in an insectary on semisynthetic diet (Seth and Sharma 2001). The adult moths were placed in pairs of four in each cage made of Perspex and nylon (size, 20 x 20 x 20 cm) for the purpose of mating. In small plastic containers cotton swabs saturated with 10% (w/v) honey was placed as adult feed. The swabs were replaced every day. The leaves of castor were put in each cage to serve as ovipositional trap. The eggs were collected, surface sterilized and kept for incubation in high RH (80%). The larvae were reared on semi-synthetic diet till pupal stage, after which the eclosed adults were placed in the cages for pairing to continue the lifecycle.

For the present study, 0-1 day old adult females were exposed to gamma radiation doses (130 and 200 Gy) at a dose rate in the range of 0.625-0.429 KGy/hr in a Co^{60} research irradiator, Gamma Chamber 5000 (GC 5000) located at Institute of Nuclear Medicine and Allied Sciences (INMAS) of Defence Research and Development Organization (DRDO), Delhi-110054. For control, the non-irradiated female moths were exposed to the same conditions (light, temperature etc.) as the irradiated females except the radiation exposure. The relative gene expression study for pheromone biosynthesis activating neuropeptide (PBAN) was conducted by excising the head and upper thorax (brain+ SOG complex) of the adult female moths (treated and untreated). The sampling was conducted under different radiation doses (0, 130, 200 Gy) and two light (photophase and scotophase) regimens. The sampling was performed 4-6 hr after onset of photophase and scotophase as per the respective regimen.

Total RNA was isolated from the above tissues using Trizol reagent followed by phenol-choloroform extraction method and treated with DNase I. The RNA concentration in each sample was measured through NanoDrop 2000 C (Thermo Fischer Scientific). 1 µg of pure RNA treated with DNase was used for single stranded cDNA synthesis by Revert Aid First Strand cDNA synthesis kit following manufacturer's protocol. Gene-specific direct primers were designed for *PBAN* gene. FASTA sequence for mRNA obtained from NCBI and online program of primer quest tool was used to

design the primers and used for qPCR (quantitative real-time PCR). The primer used had a forward sequence 5'- CTCGGCAGGACGATGAATTT -3' and a reverse sequence 5'- CTGTTGGTACTCCTGACCATTC -3'. The quality and specificity of the primer was tested with the respective tissue by performing RT-PCR and the primer pair with a single melt curve peak was selected. qPCR was performed using SYBR green on Applied Biosystems ViiA7 real-time PCR system using standard run procedure. *EF1* was taken as reference gene for normalization with insect specific primers (Forward sequence 5' GACAAACGTACCCATCGAGAAG-3', Reverse sequence 5'-GATACCAGCCTCGAACTCAC-3'). The reference and gene of interest for all treatment groups were run in duplicates with each treatment group containing 6 replicates (n=6) in a single plate to avoid variations. Each 384-well PCR plate included the non-template and sample controls without reverse transcriptase, and an endogenous control of *EF1* gene (housekeeping gene). The relative expression of the targeted gene was determined by ΔΔCt method (Livak, 2001). This method used the difference in Ct value between reference and target genes to calculate an estimated fold change in the target gene. The negative of the resultant ΔΔCt value powered to 2 ($2^{-\Delta\Delta ct}$) was plotted as relative mRNA expression of the target gene. All statistical analyses were performed using Graph Pad Prism software (version 9.0), with 6 replicates, as specified in the text. One-way ANOVA, followed by Tukey's multiple comparisons was performed on each data set to analyze the differential effect of radiation on gene expression. The student's t-test was performed to compare the effect of light phases on gene expression under different radiation regimens with respect to control.

RESULTS AND DISCUSSION

F1 sterility technique for the control of a serious lepidopteran pest, *S. litura,* would usually involve the irradiation of male moths with proposed partially sterilizing gamma dose (like 130 Gy), and the radio-sterilized male moths would then compete with the wild males to mate with wild females leading to fully sterile or almost fully sterile F1 progeny and eventually pest suppression. With an aim to release both sexes simultaneously, 130 Gy (a proposed partial sterilizing gamma dose for male moths) was identified as complete (100%) sterilizing dose for female moths (Seth, unpublished) in view of differential radio-sensitivity of both sexes (female moths being more radiosensitive than male moths) (Traut,1977). The combined release program has been proposed to exclude the tedious procedure of sex-based segregation before irradiation treatment in the traditional SIT and F1 sterility technique (Kittayapong et al., 2018), but this release program should also be able to provide reproductively competent irradiated moths to the pest infested field to transmit the sterility effectively into the wild population. Therefore, it was necessary to understand the effect of female irradiation on its reproductive fitness. One of the crucial factors determining female reproductive fitness would be to produce pheromones and exercise the calling behaviour. PBAN (pheromone biosynthesis activating neuropeptide) is an important neuropeptide which triggers the biosynthesis of pheromone in the female moths.

Therefore, for the inclusion of irradiated female moths in the F1 sterility programs, it was imminent to study the effect of radiation on *PBAN* gene expression which might consequently affect the pheromone production at the time of mating. In this study, the *PBAN* gene expressed in the brain+SOG complex of female *S. litura* was quantified in scotophase and photophase with the moths exposed to different radiation doses (130 Gy and 200 Gy). The relative gene expression showed a declining trend as the radiation dose increased and the decrease was statistically significant in irradiated insects with respect to control [photophase (One way ANOVA, $F_{(2,15)} = 13.10$, $p < 0.05$), scotophase (One way ANOVA, $F_{(2,15)} = 4.69$, $p < 0.05$)] (Fig. 1). It has been seen in various nocturnal moths

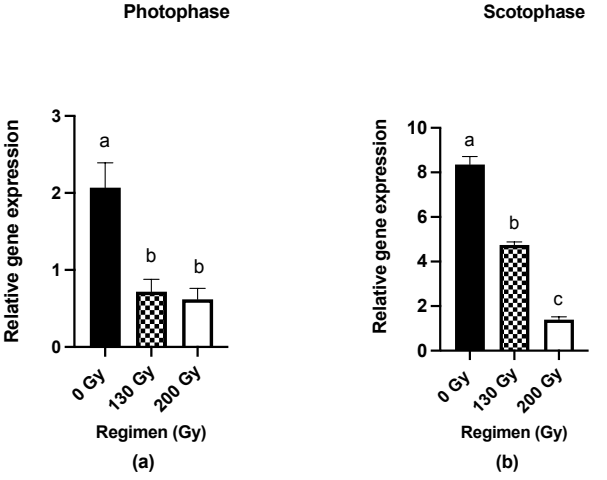

Fig. 1. *PBAN* gene expression of irradiated female *S. litura* moths in different light phases. (a) *PBAN* gene expression in photophase, (b) *PBAN* gene expression in scotophase. Means+SE followed by same letters not statistically different at $p<0.05$ (One-way ANOVA followed by Tukey's multiple comparisons)

that pheromone production which is triggered by *PBAN* gene expression is usually high at night (Levi-Zada, 2021). In this study we found similar results that the *PBAN* gene expression was higher at night (scotophase) than in the day (photophase) by female *S. litura,* that corroborated to the earlier studies. This diel rhythmicity is one of the factors responsible for maintaining the circadian rhythm of mating at night in the nocturnal insects (Zavodska et al., 2009; Bloch et al., 2013). This study showed the effect of radiation on the *PBAN* gene expression rhythmicity in the irradiated female *S. litura.* The irradiated insects showed a significantly reduced expression in photophase and scotophase in comparison to control. The diel variations of the *PBAN* gene expression was distinctly maintained in the irradiated females at 130 Gy (photophase vs scotophase, t-test, $p<0.05$) in comparison to control (0 Gy), whereas at higher dose of 200 Gy, the *PBAN* expression was markedly reduced in photophase and scotophase but diel variation was apparently noticed (Fig. 2).

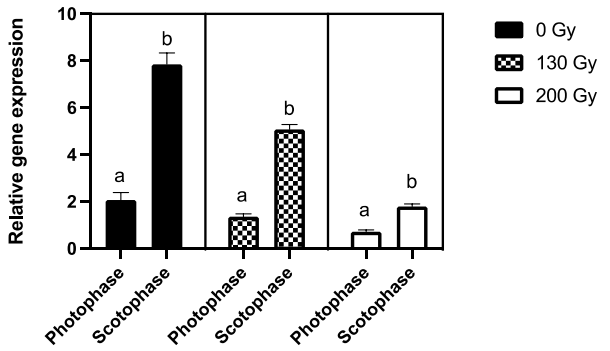

Fig. 2. Profile of *PBAN* gene expression with respect to photophase and scotophase in female moths irradiated at different doses. Means+ SE followed by different letters within each irradiation regimen statistically different at $p<0.05$. (Student's t-test for each radiation regimen comparing scotophase and photophase).

The *PBAN* gene expression observed in this study was influenced by ionizing radiation dose, and the decline in *PBAN* expression was apparent at 130 Gy but it was drastic at higher dose of 200 Gy, which validated the gradational response of gamma radiation and led to the selection of a preferable lower dose that was enough to completely sterilize the female moths. The gene expression analysis carried out in this study confirmed the diel rhythm of *PBAN* expression, and its predominant expression in scotophase corroborated with nocturnal behaviour. The current study provides an insight into the photosensitive expression of *PBAN* gene under different light regimen. In *S. litura,* the onset of mating was during the scotophase and the mating activity was not observed in the photophase. This rhythmicity of mating was also apparently reflected in the premating behavior that included the process of pheromone production, its release and male moth activation, followed by mating during the scotophase. These findings support the use of 130 Gy for complete sterilization of the female moths wherein *PBAN* gene expression profile observed was indicative of the circadian rhythmicity of the same, although the relative gene expression was less than control. This study on *PBAN* expression and its diel variations vis-a-vis control supported the reproductive competence of radio-sterilized female moths (130Gy) and their employment in the simultaneous release of both irradiated sexes in F1 sterility technique.

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AUTHOR CONTRIBUTION STATEMENT

RKS conceived and designed research. MS conducted experiments. MS, NA and NV assisted in the conducting experiments and validation of data. RKS, MS analyzed the data and involved in writing, editing and reviewing the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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CULTIVABLE GUT MICROBIAL DIVERSITY OF IRRADIATED *SPODOPTERA LITURA* (F.)

Chandra Kant Singh, Kushneet Kaur Sodhi[1], Priya Yadav and R K Seth*

Applied Entomology and Radiation Biology Unit, Department of Zoology,
University of Delhi, Delhi 110007, India
[1]Department of Zoology Hansraj College, University of Delhi, Delhi 110007, India
*Email: rkseth57@gmail.com (corresponding author): ORCID ID- 0000-0001-7873-1362

ABSTRACT

The present study was aimed to ascertain the effect of male parent irradiation (in context of radio-genetic 'Inherited sterility technique' (IS), for *Spodoptera litura* suppression) on the cultivable gut bacteria of its F1 progeny of irradiated males (100Gy, 130Gy) in relation to control using culture dependent method. The F1 progeny were subjected to isolation of culturable microbes and 16S rDNA sequence-based identification. The Scanning electron micrographs of the F1 larval guts showed the presence of biofilms associated with bacteria, as a manifestation of stress response unlike in control. The bacteria in the control were found to belong to the genera *Bacillus, Pseudomonas,* and *Enterococci,* whereas in the irradiated F1 progeny *Bacillus* and *Pseudomonas* were predominantly present. Biochemical characterization and antibiotic tests of the bacteria showed a differential pattern in F1 progeny than in control. The bacterial abundance was increased due to irradiation stress, whereas the generic richness was apparently more in control than in irradiated regimens.

Key words: Culture dependent bacteria, 16SrRNA gene sequencing, *Spodoptera litura,* insect irradiation, microbiota, phylogenetic analysis, biochemical characterization, antibiotic tests

Microbes have an important role in the overall growth and development of insects. A panoply of microbial population inhabits the insect gut which can be either existing mutually with the host or in an obligatory relationship (Gupta and Nair, 2020). Endosymbionts have been found to impact the insect's fitness along with playing major role in the supply of essential nutrients, production of pheromone, and aids in digestion and reproduction (Singh et al., 2021). Aphids and termites which feed on a specialized niche are particularly important because of the biochemical transformation that takes place in the enzymes of the microbes (Prasad et al., 2018). The larvae of lepidopterans are mostly herbivores and the food bolus are not sterile (Dillon et al., 2004). The native gut bacteria of the insects detoxify the harmful secondary metabolites along with protecting the host from colonization of the pathogens (Dillon et al., 2010) and maintains the homeostasis of plant defense elicitors in the lepidopteran larvae. *Spodoptera litura* (F.) also known as tobacco cut worm, is an economically serious agricultural pest that feeds on most of the plant species and is polyphagous in nature (Chunxian et al., 2004). It belongs to the Noctuidae family and has a worldwide distribution. The larvae of this pest moth are gregarious feeders during the early instar and feed by scraping the leaves. Later instars can completely devour the fruits and leaves leading to a complete loss in the crop growth (Kaur and Chandi, 2021).

Spodoptera litura is a serious pest in India and it has developed resistance against a variety of insecticides used to control it (Tong, 2013). Hence, an environment friendly radio-genetic technique, Inherited Sterility Technique (IS), as a modified 'Sterile Insect Technique' (SIT) has been proposed to control various lepidopteran pests including *S. litura* (Seth and Sharma, 2001; Seth et al 2016a, b, Marec and Vreysen, 2019). A radiation dose range of 100-130Gy was proposed as partially sterilizing dose to be used in IS technique against *S. litura*, and the moths must be biologically viable to compete with wild population and transfuse the inherited sterility to tackle this pest. In this radio-genetic technique (IS), the male moths are sterilized partially by radiation but they are supposed to be behaviourally viable, so that the inherited sterility can be employed in pest control. The reproductive viability may depend on inherent characteristics, viz., metabolism, body size, age, weight, and nutrients. Insect gut flora may govern mating behaviour along with associated reproductive activities and also aid in nutritional physiology (Saour, 2014). There are no reports related to the effect of irradiation on the structure of the Lepidopteran gut bacteriome. It is documented that the gut associated

bacterial species have a correlation with the overall ecological fitness and biological quality of their host, i.e., the insects (Cai et al., 2018). Hence, there is a need to explore the influence of irradiation on insect gut diversity which might be correlated with pest's reproductive viability. In view of this, the current study in its initial phase was aimed to ascertain the cultivable bacterial community structure and composition within the gut of the *S.litura* larvae and determine the effect of male parent irradiation on the cultivable gut microbial diversity harbouring the F1 progeny pest larva (derived treated male parents), so that the irradiation impact on gut microbial diversity of F1 progeny could be correlated with its development and reproductive competence vis-à-vis control (untreated insect larvae).

MATERIALS AND METHODS

Insect culture originated from agricultural fields around Delhi was maintained in the laboratory (27± 1°C, 75± 5% RH, 12:12 hr L:D with lights on at 06.00 hr and off at 18.00 hr) on castor leaves (Seth and Sharma, 2001). Irradiation was carried out at the Institute of Nuclear Medicine and Allied Sciences (INMAS) of the Ministry of Defense in Delhi in a Co^{60} source Gamma chamber. The radiation dose rate of the source ranged from 0.693 to 0.488KGy/h. 0- 1 day old adult male moths were irradiated at 100 or 130Gy, as proposed by Seth and Sharma (2001). The irradiated male adults (100Gy♂ and 130Gy♂) were crossed with normal females (0Gy ♀) and sixth instar larvae (L6) of the F1 progeny derived from both the 100Gy♂ and 130Gy♂ male parents were selected along with F1-L6 from normal males (0Gy♂) as control.

Culturable bacteria were isolated from the larval gut. Fifteen sixth instar larvae (L6) were collected and starved for 24 hr. 70% ethanol was used for the washing of the larvae (four times) followed by 5% sodium hypochlorite (v/v) for surface sterilization. The excess of sodium hypochlorite was then removed using the autoclaved distilled water. The larvae were dissected in 1 ml of phosphate buffer saline (pH 7.0) by removing the whole gut and then it was pooled. All the steps were carried out in a Laminar flow cabinet, following aseptic conditions (Thakur et al., 2015). The bacterial 'colony forming unit' (CFU) titre was studied in the irradiated F1 larvae (100Gy F1L6 and 130Gy F1L6) as well as in control (0Gy F1L6) gut. The homogenate was serially diluted using the phosphate buffer saline.100 µl of the dilution was spread plated on Luria Bertani agar plates (10g tryptone; 5g peptone; 5g NaCl; 15g NaCl in 1 l of MQ water). The plates were incubated in a BOD incubator at 30± 2°C and the bacterial CFUs were enumerated in the F1 progeny of irradiated male parent with respect to control by plating serial dilution on LB agar. The statistical analysis was performed using Graph Pad Prism software (version 8.0). One way ANOVA was used followed by Tukey post-hoc test for multiple comparisons between the bacterial titres (CFU/ ml) under different regimen. The colonies were differentiated based on their size, colour and morphology, and a single representative isolate of each morphotype was transferred to a new plate. After five repeated streaking and re-streaking, the purified isolates were preserved in LB agar in 70% glycerol stocks at -20°C for further study.

The gut bacteria of *S.litura* larvae was studied under scanning electron microscopy. After dissection, the entire gut was fixed by dipping in 2.5% glutaraldehyde overnight at 4°C (Wipfler et al., 2015). The next day, the samples were centrifuged for 10 min at 9000 rpm in 0.1M phosphate buffer (pH 7.0) followed by dehydration in increasing concentration of ethanol series. The imaging was done at the University of Delhi, USIC. The biochemical test was performed using KB003 Hi-media kit. With 80µl of the overnight grown bacterial cultures, followed by incubation for 24 hr at 30°C (Gandotra et al., 2018; Bhat et al., 2021). When the bacterial cultures were inoculated in the test kit, the substrate present in the kit exhibits a colour change that could be interpreted either visually or by the addition of a reagent. The isolates derived from the F1 progeny of control and irradiated parent were screened for protease and lipase production enzyme. A skim milk agar plate was used for the protease enzyme assay, wherein the bacteria were point inoculated onto the skim milk agar plates and incubated under normal conditions. The bacteria capable of producing caseinates or peptidases would degrade the protein present in the milk powder. A clearing zone was obtained indicating casein digestion (Azis et al., 2019). The tween 80/20 agar plate was used for assessing the lipase activity in the isolated bacteria. The bacteria were point inoculated onto the plates, and incubated under normal conditions. The deposition of calcium crystals was noted for 3-4 days. The isolates positive for lipolytic activity produced a prominent halo of degradation around them indicating deposition of calcium salt crystals of lauric acid (Azis et al., 2019). For catalase enzyme activity, hydrogen peroxide (H_2O_2) was used. A glass slide was used to perform this test. A small amount of bacterial colony was taken and place on the glass slide and one drop of 3% H_2O_2 was placed

onto the isolates. The positive test accounted for the formation of the bubbles which was due to the oxygen production. No bubble formation was accounted for negative reaction (Reiner, 2010).

The susceptibility of bacteria for different antibiotics were checked using Kirby Bauer test. In this qualitative test, Hi-media icosahedral antibiotic discs impregnated with known concentration of antibiotics were used. The disc was kept on the agar plate, already inoculated by the test bacteria. This disc was incubated and during that period, the antibiotics could diffuse from that icosahedral disc to the agar plate. Based on the solubility of the antibiotic and its molecular size, a concentration gradient was formed on the agar. If the organism was resistant then there would be growth around the disc and if the organism was susceptible then no growth was observed. Zone of inhibition was the area of no growth around the antibiotic disc. Using the scale, the zone of inhibition was measured (Khan et al., 2019).

DNA isolation from culturable bacteria was done using the GTE method (Glucose Tris EDTA method). Polymerase chain reaction (PCR) for the genomic DNA obtained from each bacterial isolate was set up to amplify the variable regions of the 16s rRNA genes. A forward 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and reverse 1492R (5'GGT TAC CTT GTT ACG ACT T 3') 16S rRNA primers were used. The reaction mixture of 25µl was prepared to have the concentration of 1µM for forward and reverse primer each, 25 ng DNA template, 200 µM dNTP (each), 1.5 U Taq polymerase, Taq buffer (1X), and DNase free water for making up the volume. PCR conditions followed were as initial denaturation for 5 min 94°C, followed by 30 cycles of denaturation for a min at 94°C, annealing at 55°C for one min, extension at 72°C for 2 min, and extension at 72°C for 7 min. The amplification was observed on 0.8% agarose gel with EtBr (0.5 µg ml^{-1}) in 0.5 X TBE buffer at 80 volts. PCR amplicons were identified using a 100bp DNA ladder (500µg ml^{-1}) used as a molecular weight standard marker. The gel was visualized on a UV trans-illuminator using Gel Doc (Clarridge, 2004). The amplified products were subjected to Sanger sequencing. The raw data were obtained as forward and reverse sequences. The sequences were trimmed and a consensus sequence was made using BioEdit. The obtained sequences were subjected to BLAST (Basic Local Alignment Search Tool) in NCBI/BLAST homepage (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest bacterial neighbours. The closely related bacterial species were retrieved from GenBank. MUSCLE (Multiple sequence alignment) tool was used for multiple sequence alignment in MEGA X. A neighbour-joining tree using the Maximum Likelihood method was drawn with 1000 bootstrap replicates using MEGAX software (Tamura et al., 2004; Tamura et al., 2021). One-way ANOVA followed by Tukey's multiple comparison test was performed to assess the significance among the CFU/ml in different regimens (100GyF1L6, 130GyF1L6, control). The experiment was replicated three times and a cohort of 15 larvae constituted one replicate for obtaining gut bacteria.

RESULTS AND DISCUSSION

Cultivable bacterial diversity (CFU/ml) in the F1 progeny of irradiated male parents at 100Gy and 130Gy in comparison to control when analysed revealed that the bacterial abundance i.e, the number of bacteria that could be cultured in the laboratory (bacterial count, i.e., CFU/ml), were more in the irradiated regimens (100Gy F1L6, 130Gy F1 L6) as compared to the control insect ($p < 0.05$), which indicated that in the irradiated F1 L6 gut bacterial CFU titres got increased with an increased radiation stress, the 130Gy parental irradiation had more impact than 100Gy irradiation (Fig. 1). Scanning electron micrographs in the F1 progeny of irradiated male parents at 100Gy and 130Gy in comparison to control showed that the larval guts of 100Gy F1L6

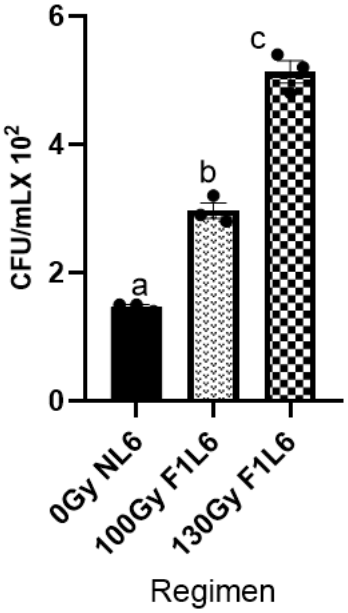

Fig. 1. Colony forming unit of bacteria (CFU/ml) in the F1 progeny of irradiated male parents at 100Gy and 130Gy in comparison to control. Bars representing Means ±SE followed by different alphabets statistically significant at $p< 0.05$ (One-way ANOVA followed by Tukey's multiple comparison test)

and 130Gy F1L6 showed the presence of a biofilm associated with bacteria in both the irradiated samples. Biofilm is generally observed in the stress conditions. The cocci shaped bacteria were distinctly visible in the control sample without any stress induced biofilm. The irradiation stress was apparent in the form of biofilm in all the irradiated regimens (Fig. 2). These biofilms are supposed to be the communities of aggregated microbial cells embedded in a self-produced matrix of extracellular polymeric substances (EPS) in response to stress (Yin et al., 2019).

Effect of irradiation on the biochemical characterization and antibiotic susceptibility in the F1 progeny of irradiated male parents and control when analysed it was observed that in the control larval gut, 36% of the biochemical enzymes were exhibited by all the four bacterial isolates, 32% of the enzymes were exhibited by ≥50% isolates, and 29% of the enzymes were not exhibited by any bacterial isolate. In the bacteria derived from the irradiated larval gut (100Gy and 130Gy), 18% of the biochemical enzymes were exhibited by all the bacterial isolates, 43% of the enzymes were exhibited by ≥50% isolates, and 40% of the enzymes were not exhibited by any bacterial isolate. Differential biochemical profiles were shown by the bacteria derived from the F1 progeny of the control and irradiated (100Gy and 130Gy) larval gut. The microbiota inhabiting the F1 progeny of the irradiated (F1) larval gut might be displaying differential metabolic activities in response to control. This might be attributed to the differential profile of biochemical enzymes exhibited by the bacterial isolates in irradiated regimens (F1 larvae) vis-à-vis control.

Bacterial inhabitants in the insect gut are of valuable significance as they increase their host survival through nutrients supplementation, by complementing the digestive enzymes or via the degradation of plant metabolites that are otherwise difficult to degrade (Gandotra et al., 2018). Insects lack a complete enzyme system and gut bacteria provide different enzymes responsible for the assimilation and digestion of nutrients (Jing et al., 2020). In this present study, the biochemical assays indicated that many of these isolates might be helping in the digestion and assimilation of nutrients of insects, Also, some bacteria might be helpful in the utilization of certain sugars. All the

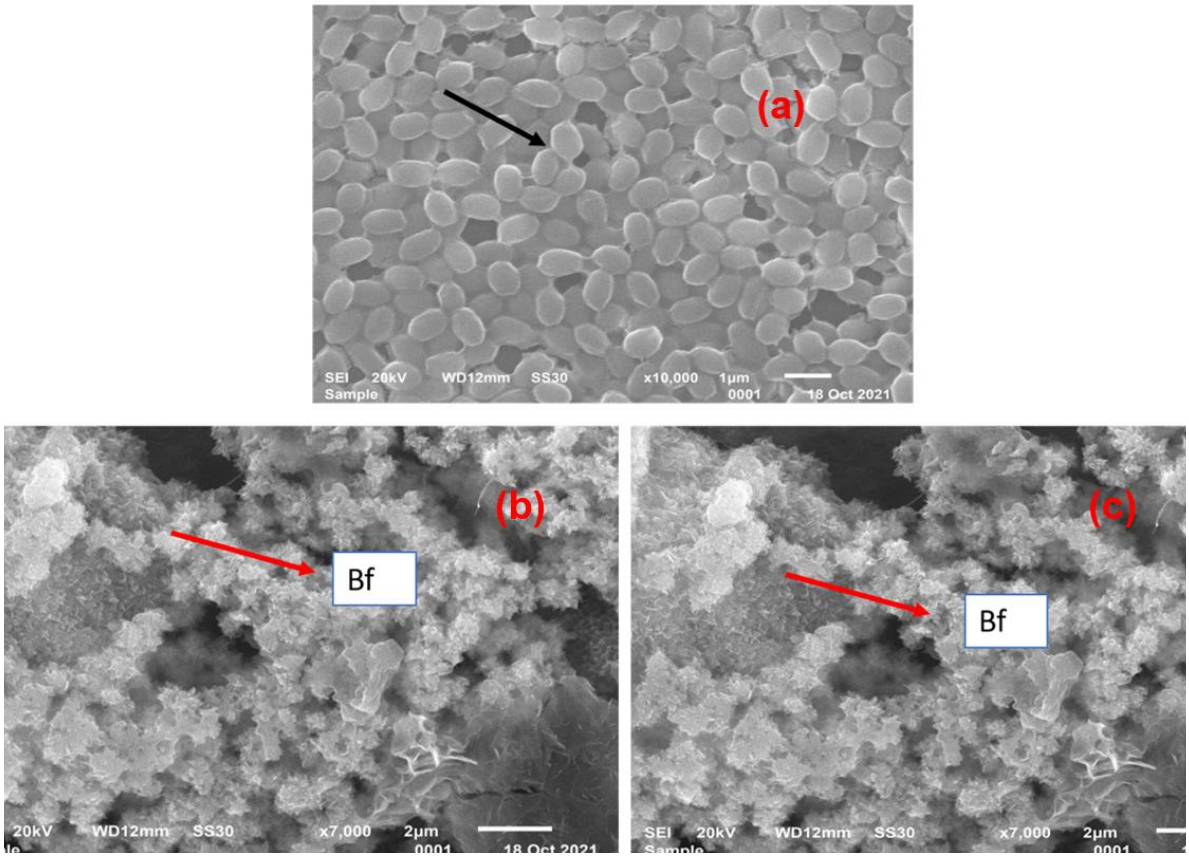

Fig. 2. Scanning electron micrographs of whole gut region of F1 progeny of irradiated male parent *S. litura,* in relation to control (a) 0Gy NL6 (Scale- 1 µm), (b) 100Gy F1L6 (Scale- 2 µm), (c) 130Gy F1L6 (Scale- 2 µm)

bacteria (isolated from irradiated regimens and control) in the present study were catalase-positive and oxidase-negative, therefore they were presumably facultative anaerobic organisms (Table 1).

Antibiotic test showed the susceptibility of bacteria derived from the F1 progeny of irradiated male parent, *S. litura,* in comparison to control. The bacteria derived from the F1 progeny of irradiated male parent, *S. litura* at 0Gy (i.e. control) showed the resistance profiles in descending order as C1> C3=C4>C2. That indicated maximum susceptibility of the C2 isolate for the antibiotics, followed by C3, C4 and C1. The bacteria derived from the F1 progeny of irradiated male parent, *S. litura* at 100Gy showed the resistance profiles as Y3>Y2>Y1. That indicated maximum susceptibility of Y1 isolate followed by Y2 and Y3. The bacteria derived from the F1 progeny of irradiated male parent, *S. litura* at 130Gy showed the resistance profiles as R2>R3>R1. The maximum susceptibility was shown by R1 isolate followed by R3 and R2. The microbiota derived from both the control and irradiated larval

Table 1. Biochemical tests of bacteria derived from F1 progeny of irradiated male parent, *S. litura* in comparison to control, to ascertain the presence of biochemical enzymes in different isolates using KB003 Hi-Media kit. (a) 0Gy NL6 (control), (b) 100Gy F1L6, (c) 130Gy F1L6

Regimen	Bacterial isolates from the Control 0Gy NL6				Bacterial isolates from the control 100Gy F1L6			Bacterial isolates from the Control 130Gy F1L6		
Biochemical test	C1	C2	C3	C4	Y1	Y2	Y3	R1	R2	R3
Oxidase production	-	-	-	-	-	-	-	-	-	-
ONPG production	-	-	-	-	-	-	-	-	-	-
Lysine utilization	-	-	-	-	-	-	-	-	-	-
Ornithine utilization	-	-	-	-	-	-	-	-	-	-
Urease production	+	-	+	-	-	+	-	-	-	+
Phenylalanine deamination	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-
Citrate utilization	+	-	-	+	-	-	-	-	-	-
Voges Proskaueuer's test	+	-	-	-	-	-	-	-	-	-
Methyl Red production	-	+	+	-	+	+	-	+	-	+
Indole production	-	-	-	-	-	-	-	-	-	-
Malonate utilization	+	-	-	+	-	-	-	-	-	-
Esculin hydrolysis	+	+	+	+	+	+	-	+	-	+
Arabinose utilization	+	+	-	+	+	+	-	+	-	+
Xylose utilization	+	+	+	+	+	+	-	+	-	+
Adonitol utilization	-	-	-	-	-	-	-	-	-	-
Rhamnose utilization	-	+	-	+	+	-	-	+	-	-
Cellobiose utilization	+	+	+	+	+	+	-	+	-	+
Mellobiose utilization	+	+	+	+	+	+	-	+	-	+
Sachharose utilization	+	+	+	+	+	+	-	+	-	+
Raffinose utilization	+	+	+	+	+	+	-	+	-	+
Trehalose utilization	+	+	-	+	+	+	+	+	+	+
Glucose utilization	+	+	+	+	+	+	+	+	+	+
Lactose utilization	+	+	-	+	+	+	-	+	-	+
Catalase test*	+	+	+	+	+	+	+	+	+	+
Protease test*	+	+	+	+	+	+	+	+	+	+
Lipase test*	+	-	+	+	+	-	+	+	-	+

*shows the tests performed using qualitative assays

gut showed different resistance profiles against the antibiotics. The microbiota in the control larval gut were more resistant to antibiotics as compared to microbiota derived from the F1 progeny of the irradiated male (100Gy and 130Gy). The bacteria derived from the irradiated larval gut showed more susceptibility towards antibiotics as they were not able to grow in the presence of antibiotics presumably because of the additional stress i.e., irradiation. In view of an overall sensitivity response of gut bacteria of normal and irradiated insects towards various antibiotics, it could be surmised that high number of isolates were susceptible to norfloxacin, gentamicin, ciprofloxacin, cefoperazonentiobiotic, and chloramphenicol. These antibiotics showed profound effects on the gut microbiota composition as high number of isolates were found to be sensitive to these drugs (Table 2).

The 16S rRNA gene amplification was carried out and approximately 1.2-1.5 kb 16S rRNA band was observed in 0.8% agarose gel. The bands were processed for Sanger sequencing. The sequenced reads were subjected to NCBI BLAST and EZ Taxon to check for the phylo-genetically similar isolates. Both the databases, viz., NCBI BLAST and EZ Taxon, are curated and were used. The phylogenetically related strains were selected and subjected to multiple sequence alignment using MUSCLE (alignment tool) in MEGA X (Software used for multiple sequence alignment and phylogenetic tree construction). A neighbor-joining tree was constructed using the Maximum Likelihood method with 1000 bootstraps (Fig. 3). The identification of the bacterial isolates were based on the 16S rRNA gene sequencing. 16S rRNA sequencing could only identify upto the genus level. Figure 3 shows the phylogentic

Table 2. Antibiotic tests of bacteria derived from F1 progeny of irradiated male parent, *S. litura* in comparison to control to ascertain the relative susceptibility or resistance of bacterial isolates towards various antibiotics. (a) 0Gy NL6 (control), (b) 100Gy F1L6, (c) 130Gy F1L6

Antibiotic tests			F1 progeny of control 0Gy NL6				F1 progeny derived from 100Gy			F1 progeny derived from 130Gy		
Antibiotics	Symbol	Conc (mcg)	C1	C2	C3	C4	Y1	Y2	Y3	R1	R2	R3
Norfloxacin	NX	10	S	S	S	S	S	S	S	S	S	S
Gentamicin	GEN	10	S	S	S	S	S	S	S	S	S	S
Chloramphenicol	C	30	S	S	S	S	S	S	S	S	S	S
Cefuroxime	CXM	30	R	S	S	S	R	S	R	R	R	S
Ciprofloxacin	CIP	5	S	S	S	S	S	S	S	S	S	S
Cefoperazone	CPZ	75	S	S	S	S	S	S	S	S	S	S
Ceftazidime	CAZ	30	R	S	S	S	S	R	R	S	R	R
Roxithromycin	RO	30	R	S	R	R	S	S	S	S	S	S
Clarithomycin	CLR	15	S	S	R	R	S	S	R	S	R	S
Co-Trimoxazole	COT	25	R	R	R	R	S	S	R	S	R	S
Netillin	NET	30	S	S	S	S	R	S	S	R	S	S
Cefaclor	CF	30	R	S	R	R	S	S	S	S	S	S
Cefotaxime	CTX	30	R	S	S	S	R	R	R	R	R	R
Cefadroxil	CFR	30	R	S	S	S	S	R	R	S	R	R
Azithromycin	AZM	15	R	R	S	S	S	S	S	S	S	S
Ampicillin/ Cloxacillin	AX	10	R	S	S	R	S	R	R	S	R	R
Penicillin	P	10	R	S	R	R	S	S	R	S	R	S
Amikacin	AK	30	S	R	S	S	S	S	S	S	S	S
Sparfloxacin	SPX	5	S	S	R	S	S	S	S	S	S	S
Ampicillin/ Sulbactam	A/S	10/10	R	R	R	R	S	R	S	S	S	R

analysis of the isolates with their closest relatives. Based on 16S rRNA gene sequencing the strain C1 showed 97.74% similarity with the genus *Bacillus* (Phylum Firmicutes), C2 showed 91.68% similarity with genus *Pseudomonas* (Phylum Proteobacteria), C3 showed 95.81%similarity to genus *Enterobacter* (Phylum Proteobacteria), and C4 showed 100% similarity to genus *Bacillus* (Phylum Firmicutes).

In the gut bacterial isolates of F1 L6 derived from the

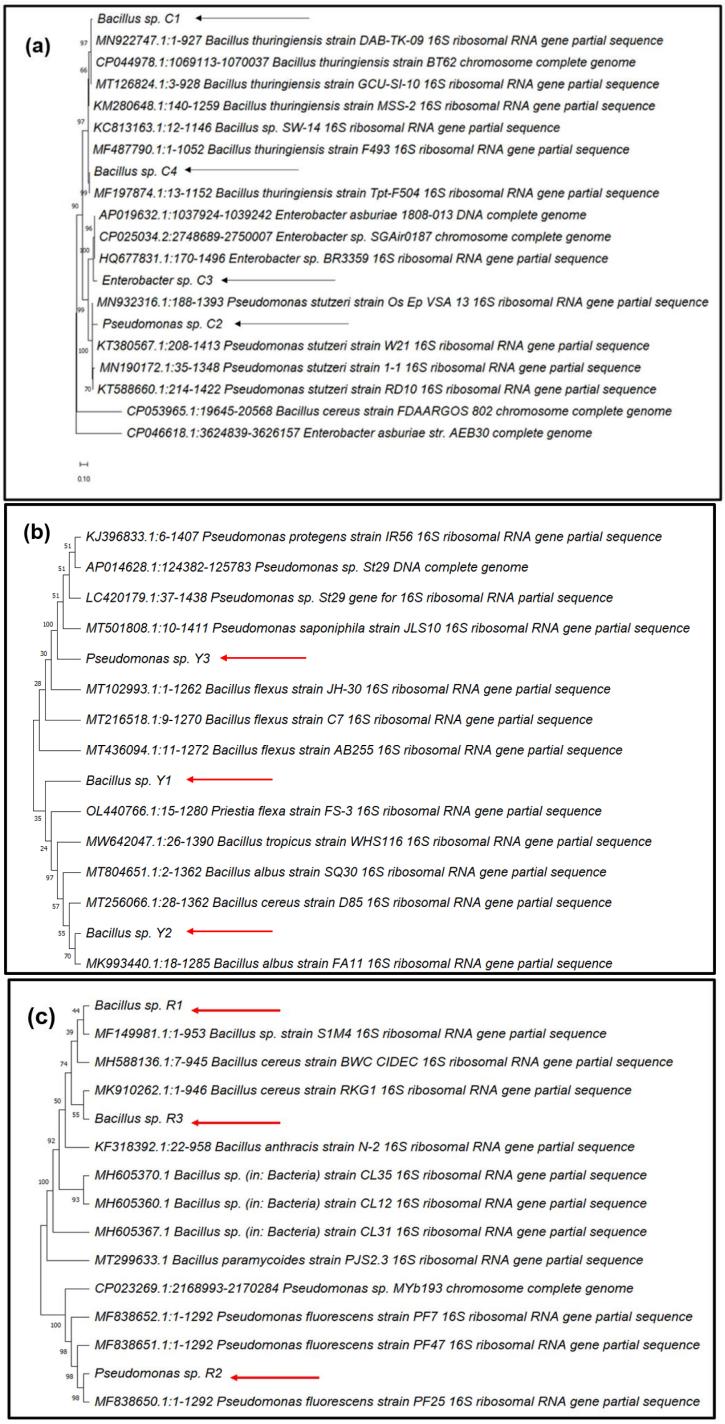

Fig. 3. Phylogenetic tree of bacteria derived from F1 L6 progeny of irradiated male parent, *S. litura* in relation to control (a) 0Gy NL6, (b) 100Gy F1L6, (c) 130Gy F1L6, using MEGA X and Neighbor joining tree (maximum likelihood method) with Bootstrap value 1000.

irradiated male parent 100Gy and 130Gy, Y1 and Y2 (100Gy regimen) showed 99.62% and 86% similarity to the genus *Bacillus*, and Y3 showed 93.06% similarity to *Pseudomonas*. Whereas R1 and R3 (130Gy regimen) showed 100% and 95.73% simlarity to *Bacillus*, and the R2 showed 93.25% similarity to *Pseudomonas*. The bacterial isolates had less similarity with already known bacterial strains in the NCBI suggesting that they could be novel bacterial isolates. Overall, it could be surmised that the bacteria in the control (0Gy) might belong to the genera *Bacillus*, *Pseudomonas,* and *Enterococcus*, whereas *Bacillus* and *Pseudomona*s were predominantly present in irradiated regimens (100Gy and 130Gy) unlike *Enterococcus* which was categorically identified in control. *Bacillus* is a Gram-positive bacteria that belong to the Phylum Firmicutes whereas *Pseudomonas* is a Gram-negative bacteria that belongs to the Phylum Proteobacteria. It is apparent from our findings that bacteria from the phylum Firmicutes and Proteobacteria were predominantly present in the Lepidopteran larval gut. The previous studies also reported that Bacillaceae and Enterobacteriaceae dominated culturable gut bacteria in the Lepidopterans. These symbionts might be contributing greatly to the physiology of these insects. A study by Hui et al. (2010) also reported that gut bacteria *Enterobacter* and *Enterococcus* isolated from lepidopteran aided in maintaining the gut pH and also enhanced the alkalinity which might be playing a critical role in tannin digestion.

In the present study, the isolated bacteria might aid in the detoxification of tannins in castor leaves (*Ricinus communis*) the primary food plant for *S. litura* which contained about 20% tannin content (Tang et al., 2012). In silkworm, *Bombyx mori* the bacteria phylum Firmicutes and Proteobacteria were reported to dominate (Liu et al., 2020). The genus *Enterococcus* was observed to be prevalent in the gut of polyphagous insects (Chen et al., 2016). The present findings corroborate earlier reports concerning these microbial groups. The bacterial isolates had less similarity with already known bacterial strains in the NCBI suggesting that they could be novel bacterial isolates. Differential behavior of the microbiota for the biochemical assays was observed in the F1 larval guts (derived from irradiated male parents) as compared to the control. Microbial CFUs titres showed that the number of cultivable bacteria were observed to be more in abundance in the irradiated regimens (100Gy F1L6, 130GyF1L6), the Genus *Enterococcus* was not identified in irradiated L6 in the present study. The bacterial abundance was found to be increased significantly due to irradiation stress, whereas the generic richness was apparently more in control than in irradiated regimens. Thus, as per the present study, the irradiation had an impact on the overall microbial diversity in the larval gut, and the observed changes in the bacterial diversity in F1 progeny of irradiated male parents might be considered as an attributing factor to induced sterility and competence of F1 moths that would be employed in IS technique for *S. litura* suppression. As only about 1% of the total bacteria were cultivable so it could not be feasible to determine the exact diversity present in the gut of the irradiated compared to the normal through the culture-dependent approach; hence, the further studies on culture independent bacteria are in progress to ascertain the bacterial diversity in irradiated Lepidopteran gut with respect to control.

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AUTHOR CONTRIBUTION STATEMENT

RKS conceived and designed research. CKS conducted experiments. CKS, KKS and PY assisted in the conducting experiments and validation of data. RKS, CKS and KKS analyzed the data and involved in writing, editing and reviewing the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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FUNCTIONAL CAPABILITIES OF SPIDERS IN SUPPORT OF SUSTAINABLE AGRICULTURE

Somanath Sahoo*, Gitanjali Mishra, Jaya Kishor Seth, Laxman Kumar Murmu and Santhosh Goud

Post Graduate Department of Zoology, Berhampur University, Berhamour 760007, Odisha, India
*Email: cool.somanath@gmail.com (corresponding author)

ABSTRACT

Modern agricultural techniques have a detrimental effect on ecosystems, and hence ecofriendly agriculture is required for sustainable agriculture with biodiversity conservation. Spiders play a vital role in keeping pest populations under control as an alternative to chemical pesticides. This study assessed the functional diversity of spiders to control pest populations by estimating their diversity in diurnal and seasonal appearance, preferred microhabitats, and hunting strategies. From August 2020 to November 2021, random quadrate sampling was undertaken in cultivated and wild plant communities. A metaanalysis of the ecology and diversity found that spiders have the capability to provide biological pest control against a wide variety of pests in Odisha's coastal environment.

Key words: Diversity, spiders, agricultural pests, sustainable development, organic farming

Agricultural output is the basis of a nation's economy, providing humankind with the ingredients it needs for survival and raw materials for industrialisation (Praburaj et al., 2018). After independence, widespread and intensive farming practices have brought a green revolution in India (Parayil, 1992). On the other hand, the continuous conversion of forestland to farmland and the application of artificial chemical fertilisers and pesticides raises issues about soil health, pollution, pesticide toxicity, and agricultural production sustainability (Yadav et al., 2013). So, to have sustainable agriculture, organic farming could be an appropriate option where we reduce the application of harmful chemicals while enhancing the use of organic compost to retain natural nutrient recycling (Mahdi et al., 2010; Pindi and Satyanarayana, 2012) and augment the effectiveness of natural enemies to control pests (Letourneau and Bothwell, 2008; Garratt et al., 2011; Xu et al., 2011; Puech et al., 2014). The principal loss of agricultural productivity is caused by various insect pests and mites that cause damage to different parts of the crop plants (Bellotti and Schoonhoven, 1978; Dhaliwal et al., 2015; Rathee and Dalal, 2018). To control those pests, it is, therefore, necessary to have a sufficient diversity of natural enemies with different microhabitats, activity periods, population dynamics, and hunting strategies with high fecundity and dispersal capabilities. All these characteristics are fulfilled by spiders (Sunderland, 1999). Again, ecofriendly agriculture must enable existing natural enemies to resist pests while also boosting agricultural production via locally accessible conventional approaches that cause little disruption to the natural environment (Pierce and Nowak, 1999). Naturally, spiders occupy all kinds of microhabitats and ecological niches to enhance agricultural productivity. Integrating biodiversity conservation by conserving the spider fauna, which is considered the king of microhabitats, with agriculture is the most contemporary and environmentally beneficial strategy for achieving sustainable agriculture, effective use of natural resources, low management effort, and the development of ecotourism (Dumanski et al., 2006; García-Frapolli et al., 2007; Norris, 2008). This research aimed to determine if naturally occurring diverse fauna of spiders could manage agricultural pests by studying their diversity in terms of diversity indices, occupied microhabitat, feeding behaviour, and diurnal and seasonal appearance in the coastal belt of Odisha.

MATERIALS AND METHODS

From August 2020 to November 2021, random quadrate sampling was conducted in cultivated and wild plant communities. Cultivated plant communities include rice, pulses, vegetables, cashew and mango plants. For low height herbs and shrubs, $1\times1m^2$ quadrate and for high foliage shrubs and trees $5\times5m^2$ quadrate (as plants were distributed sparsely) with a maximum sampling height of 6ft were used to collect spiders from various microhabitats at four time periods (early morning, midday, afternoon and dusk hours) of the day and four seasons (premonsoon, monsoon, postmonsoon and winter) of the year. From each time period of a season, at least 5 quadrates were taken from each plant community. The study was carried out in

the coastal areas of Gopalpur and its surrounding area in Odisha, India (19° 15.149'N, 84° 53.554'E). Only mature individuals were considered for estimating diversity. Field observation, calculation and sampling of specimens were done using sweep nets, beating and shaking branches, leaf litter collection, pitfall traps, and handpicking. Following the collection of predaceous arthropods, photographs were taken in the laboratory once again, and specimens were preserved in a separate container with proper abelling for further identification. The identification was done using key characters in Jocque and Dippenaar-Schoeman (2007) and Tikader (1987), Tikader and Malhotra (1980), World Spider Catalogue (2022). Statistical analysis was done using PAST (Paleontological Statistics), version 4.08. The functional diversity of predaceous arthropods to control agricultural pests was estimated by calculating the number of species and individuals sampled from different microhabitats, bearing different feeding habits and appearances in different periods of the day and seasons.

RESULTS AND DISCUSSION

The taxonomic diversity of spider fauna depicted in Table 1 reveal 37 species belonging to 10 families and 30 genera (Fig.1). The maximum samples belonged to *Scytodes thoracica*, followed by *Anepsion maritatum*, *Myrmarachne* sp., *Peucetia viridana*, *Cyclosa insulana*, *Hyllus semicupreus*, *Telomonia dimidiata*, *Olios melliti*, *Myrmaplata platleoides*, *Phintella vittate, Oxyopes javanus* etc. In wild vegetation, *Anepsion maritatum*, *Cyclosa insulana*, *Gastracantha geminatum*, while in cultivated vegetation, *Scytodes thoracica*, *Phintella vittate* and *Hyllus semicupreus* were dominant species. The species richness and total abundance of predaceous arthropods in the wild plant communities were 37 and 707 whereas in cultivated plant communities that was 29 and 304, respectively (Table 1). The computed diversity indices (Simpson's diversity indices (D, 1-D), Shannon's diversity index (H), Evenness (e^H/S), Brillouin index, Margalef index, Equitability (J), Berger-Parker index, Fisher's index), revealed that although the total abundance of predaceous arthropods in cultivated lands was less than half of that of wild vegetation, still the whole diversity is not greatly different between them. This result indicates that chemical fertilisers and pesticides and even burning action in the field after harvesting cause a severe decline in the spiders (Pekar, 2012). Diversity of spiders increased due to the quicker migration of a wide number of species (agrobiont spiders) from wild plant communities to cultivated plant communities (Samu and Szinetar, 2002).

Table 2 shows the diverse functional capability of the spider to control agricultural pests. The number of

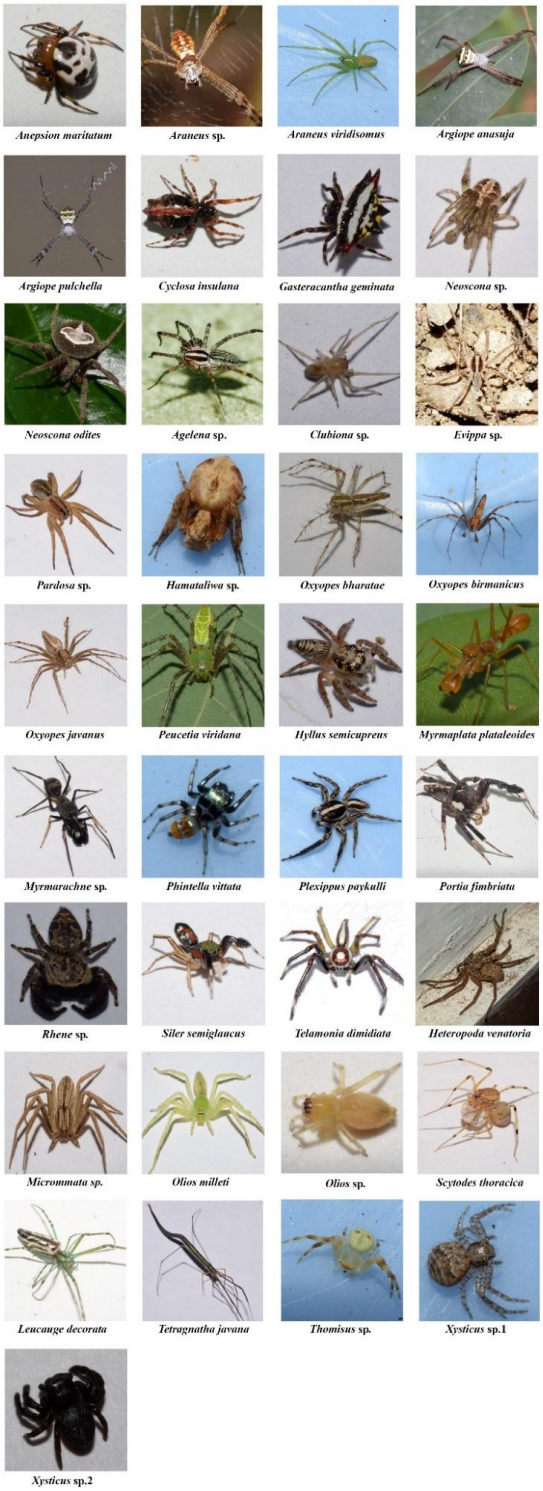

Fig. 1. Spider diversity (Gopalpur, Odisha)

Table 1. Diversity of spider fauna (wild and cultivated plant communities)

Families	Species name	No. of individuals sampled from wild habitat	No. of individuals sampled from cultivated habitat	Total No. of individuals sampled
Araneidae (Orb web or tent web spiders)	*Anepsion maritatum* (O. Pickard-Cambridge, 1877)	48	11	59
	Araneus sp.	17	2	19
	Araneus viridisomus Gravely, 1921	8	0	8
	Argiope anasuja Thorell, 1887	17	11	28
	Argiope pulchella Thorell, 1881	22	17	39
	Cyclosa insulana (Costa, 1834)	38	13	51
	Gasteracantha geminata (Fabricius, 1798)	37	7	44
	Neoscona sp.	8	1	9
	Neoscona odites (Simon, 1906)	1	0	1
Agelenidae (Funnel web spiders)	*Agelena* sp.	12	9	21
Clubionidae (Sac spiders)	*Clubiona sp.*	14	11	25
Lycosidae (Wolf spiders)	*Evippa* sp.	12	6	18
	Pardosa sp.	16	9	25
	Hamataliwa sp.	5	0	5
Oxyopidae (Lynx spiders)	*Oxyopes bharatae* Gajbe, 1999	18	13	31
	Oxyopes birmanicus Thorell, 1887	13	11	24
	Oxyopes javanus Thorell, 1887	35	9	44
Salticidae (Jumping spiders)	*Peucetia viridana* (Stoliczka, 1869)	36	19	55
	Hyllus semicupreus (Simon, 1885)	29	21	50
	Myrmaplata plataleoides (O. Pickard-Cambridge, 1869)	29	17	46
	Myrmarachne sp.	35	23	58
	Phintella vittata (C.L. Koch, 1846)	26	18	44
	Plexippus paykulli (Audouin, 1826)	12	2	14
	Portia fimbriata (Doleschall, 1859)	9	1	10
	Rhene sp.	3	0	3
	Siler semiglaucus Simon, 1901	3	1	4
	Telamonia dimidiata (Simon, 1899)	36	14	50
Sparassidae (Giant crab spider)	*Heteropoda venatoria* (Linnaeus, 1767)	5	3	8
	Micrommata sp.	9	0	9
	Olios milleti (Pocock, 1901)	31	19	50
	Olios sp.	10	2	12
Scytodidae (Spitting spiders)	*Scytodes thoracica* (Latreille, 1802)	36	29	65
Tetragnathidae (Long jawed spiders)	*Leucauge decorata* (Blackwall, 1864)	26	3	29
	Tetragnatha javana (Thorell, 1890)	11	0	11
Thomisidae	*Thomisus* sp.	13	2	15
	Xysticus sp.1	19	0	19
	Xysticus sp.2	8	0	8

Table 2. Functional capabilities of spiders and their variation

Name of the ecological characters	Types	No. of Species	No. of individuals
Occupied microhabitats	Leaves and branches of tree canopy or high shrubs	13	359
	Bark of trees	2	15
	Inside leaf litter	2	43
	Inside low shrubs and herbs	21	801
	Inside small herbs and grasses on the ground	3	50
	On barren or rocky land	3	42
Feeding behaviour	Passive hunter by making webs	13	446
	Ambush hunter	16	510
	Active hunter	8	354
Activity period in a day	Early morning (Sunrise-10am)	25	489
	Midday hours (10am-2pm)	22	434
	Afternoon hours (2pm-6pm)	9	136
	Dusk hours (After Sunset)	12	251
Seasonal appearance	Premonsoon (March-May)	27	511
	Monsoon (Jun to Aug)	19	286
	Postmonsoon (Sep-Nov)	25	392
	Winter (Dec-Feb)	11	121

species and individuals of spiders was highest inside low shrubs and herbs, which can provide the best biological protection from pests to the annual crop plants having low foliages like rice, pulses, vegetables, etc. The reason for having the highest species diversity inside low shrubs and herbs was due to frequent storms in that coastal area which cause the wild vegetation community to possess mostly herbs, shrubs, and saplings of some trees with sparsely distributed low height trees. Most of the species used ambush hunting strategies to get their prey. Again the diversity of web-building spiders is the potential to control a wide variety of flying adult insect pests, as webs can be traped and kill pests which might be or might not be their prey. Active hunting also killed several active pests but is not significant strategies in cultivated communities as most of the agricultural insect pests were slow-moving. Most of the species were active during morning hours (Sunrise to noon). After that, their number declined, and again towards the sunset, both the number of species and individuals increased. Most of the species sampled were diurnal. Some species collected from tree bark, under the leaves, leaf litter and stones were collected during their resting condition as they were nocturnal in their behaviour. As different species were active during different hours of the day including nocturnal species, the spider community can able to control all sorts of agricultural pests. The number of species and individuals was highest during the premonsoon season followed by monsoon, postmonsoon and winter seasons. Wide varieties of pulses and vegetables were usually harvested during the premonsoon season (Singh, 2013) and that is the appropriate season to control the diverse pest population in crop fields. During the early and late winter season, we sampled 11 species to be active and breed during that time. In the winter season, spiderlings were distributed through ballooning ways to reach the cultivated land easily. During the winter season, the number was poor because most of the sampled species were juveniles and were not considered to estimate the diversity.

It is suggested that having wild vegetation around the cultivated land is essential to preserve spider diversity even after disturbances in cultivated areas due to the harvesting and application of chemical pesticides. In integrated pest control strategies, when required low dose of chemical pesticides can be applied to the core area of the cultivated plants, after which the spider species can quickly eat the resistant pests to control their destructive activities. Moreover, switching from conventional agriculture to ecofriendly organic farming is crucial for sustainable development in the agricultural sector.

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AUTHOR CONTRIBUTIONS

S Sahoo conducted the sampling, collected, took the photographs and identified the specimens. S Sahoo, G Mishra, J K Seth, L K Murmu and S Goud made an analysis and prepared the manuscript. All authors read and approved the manuscript.

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EFFICACY OF TWO-IN-ONE MODEL TRAPS AND SUN DRYING AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) IN MUNGBEAN

R Kooner*, D K Sharma and K S Suri

Department of Entomology, Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: rubalsidhu05@pau.edu (corresponding author)

ABSTRACT

Detection of insect infestations is essential for quality assurance and helps in locating insect infestations, for early diagnosis of low-level infestation and to ascertain the success of control measures. This study evaluates the efficacy of two-in-one model trap against cowpea bruchid *Callosobruchus maculatus* (F.) in stored mungbean in six months of storage. Tamil Nadu Agricultural University (TNAU) developed two-in-one model traps were used. The observations on number of adult beetles trapped, % weight loss and grain damage were recorded at the end of each month, for six months. The results obtained conclude that use of one trap and sun drying of mungbean grains for five days is significantly effective in reducing the number of adult beetles. No weight loss and grain damage were recorded with this treatment, where only one trap/ container was used followed by sun drying for five days at the end of fourth month. Similar results were obtained up to six months. The % weight loss of 1.75 and 4.00% grain damage observed at the end of sixth month with one trap and three day sun drying of grains exhibit its efficacy.

Key words: *Callosobruchus maculatus*, grain damage, two-in-one model trap, grain damage, weight loss, mungbean, pulse beetle, losses, months, sun drying

Among pulses, mungbean, *Vigna radiata* (Linn.) Wilczek is important (Yadav et al. 1994), and traders mostly store these at least for few months. During this storage, pulses suffer enormous losses due to pulse beetle (Fletcher and Ghosh, 2002), the infestation of which starts in the field and subsequently carried to the stores (Khan et al., 2015). Amongst these pulse beetles, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), also known as cowpea dhora, is a major pest (Park et al., 2003), and cause 87 to 100 % loss within a storage period of three to six months (Akinkurolere et al., 2006; Ojiako and Adesiyun, 2013). The grains are made unfit for human consumption (Atwal and Dhaliwal, 2005). Mungbean *Vigna radiata* is the most common host for *Callosobruchus* spp., in respect of oviposition, adult emergence (66.11-70.29%) and caused 50.37 - 57.58 % grain damage in storage (Ali et al., 1999). Owing to the hazards due to pesticides, several non-chemical/ non thermal strategies are used for disinfestations of stored grains (FAO, 2017). These provide ecofriendly alternatives (Ramazeame et al. 2012), and such alternate strategies are required for timely detection and management of stored grain pests (Martinez et al. 2000; Reed et al. 2001; Shadia and Aziz, 2011). Monitoring tools such as insect probe trap, pitfall trap, and two-in-one trap developed by Tamil Nadu Agricultural University (TNAU) were analysed for the management of pests of stored grains. These have been found effective. The present study is to establish the simple and easy method to detect the infestation of internal stored grain insects so as to forecast the risk of possible pest outbreak that may indicate the need for timely control strategies or their validation.

MATERIALS AND METHODS

Present study was carried out during 2018-19 in the Post-harvest Technology Laboratory, Department of Processing & Food Engineering, PAU, Ludhiana. For maintaining the stock culture of *C. maculatus*, sound and fresh grains of mungbean variety SML-668 (Punjab Agricultural University recommended variety) were sterilized in an oven at 55^{0}C for 4 hr before start of experiment (Mookherjee et al., 1968). Then freshly emerged adults of *C. maculatus* were taken from pure culture and released on sterilized mungbean grains in battery jars, covered with muslin cloth and fastened with rubber bands and these jars were kept at under room temperature for further multiplication of the insects. During the period of study (six months), weekly mean maximum and minimum temperature ranges were 29.9-42.1 ^{o}C and 16.5-28.1 ^{o}C, respectively during 2018. During 2019 the maximum to minimum temperature ranges were 30.0-43 ^{o}C and 18.1-28.5 ^{o}C. The relative humidity varied from 21-80 % during both

the years (2018 & 2019). Fresh grains of mungbean were provided periodically for the development of beetles and the culture so maintained was further used for undertaking various investigations. The experiment was conducted in completely randomized design. There were total four treatments and each treatment had eight replications.

For conduct of experiment, five kg of sterilized mungbean grains (Variety SML-668) were taken in the plastic container and twenty pairs of freshly emerged adults (2d old) of *C.maculatus* were released into these containers. For getting freshly emerged adults, petri plates containing fresh grains of mungbean were placed in the culture jars and removed after 24 hr. These petri plates containing freshly laid eggs were separated from the culture jars and such grains were placed in different containers. The freshly emerged adults from these containers were used for the studies. One week period was given for uniform multiplication of these pulse beetles in the mungbean grains. After one week, the released beetles were removed from the container and two-in-one model trap was placed in it. The containers were covered with muslin cloth. For treatment 1, after one month, trap was removed from the container and the number of adults trapped in the funnel of trap was counted. After taking the other observations on grain damage and weight loss, the trap was placed back into the container. This procedure was repeated for six months. In second treatment, after 30 days, the observations were taken as above and sun-drying of the grains (to kill the immature stages of pulse beetle) was done for 3 days and then trap was placed back into the container.

For sun drying, the grains were placed on tarpaulin sheet in the bright sunlight for five hours and then the grains were put back into the container. The sun drying was practiced at the end of each month continuously for 3 days. Similarly, in treatment 3, the sun drying was done continuously for 5 days. The temperature range for the sun drying period was 42.1- 43 ^{o}C. All the observations were taken at monthly intervals. The following treatments were evaluated: The observations on number of adults trapped were taken at the end of each month, the trap was removed from the grains and the number of adults trapped in the funnel of the trap, attached at the end, was observed. The % weight loss was calculated using Count and Weight method (Adams and Schulten, 1978). One thousand grains were taken randomly from the sample. The number of insect damaged and undamaged grains was counted and their weight was taken on an electronic weighing balance. The % grain damage was calculated from healthy (without holes) and insect damaged grains separated on thousand grain count basis. The data pertaining to different observations were subjected to Analysis of Variance using statistical software SPSS v 20.0 (SPSS, 2011). The comparison of means was done using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

After one month, number of adults trapped in storage container with one trap followed by sun drying of mungbean grains for three days (47.28/ 5kg) was statistically at par with adults trapped in storage container with one trap and sun drying followed for five days (50.17/ 5kg) (Table 1). The efficacy of TNAU developed two-in-one model trap for timely detection of bruchids in stored pulses was evident from the recorded lowest grain damage (5.5%) in the trials conducted at farmers level (where these traps were used) as compared to 13.5% grain damage with farmer's practice (without the use of traps) (Mohan, 2014; Debebe et al., 2008). At the end of third month, statistically lower number of adults (4.00) were trapped in storage container with one trap and sun drying for five days (T_3). The mean number of cigarette beetle, *Lasioderma serricorne,* adults trapped, increased with increase in the number of traps per bag stack (Rajesh et al., 2015). T_3 continued to perform better till the end of storage period with no entrapment of adults. The data on % weight loss of mungbean grains given in (Table 1) inferred those two treatments where one trap and sun drying of mungbean grains for three days (T_2) and one trap and sun drying of grains for five days (T_3) were statistically similar. Sun drying destroys existing insect pests and their different stages present in/on the grains (Debebe et al., 2008) It also helps to reduce spoilage and enhance the dormancy period of grains (Kumar and Singh, 2013). After four months, no weight loss of grains was observed in T_3 and it was statistically better as compared to all other treatments. Maximum % weight loss was observed in untreated control (20.00%). Similar trend was observed w.r.t. all treatments.

The data on % grain damage presented in Table 1 show that grain damage among different treatments after one month of storage ranged from 8.32 to 9.60. The grain damage (%) in storage container with one trap followed by sun drying of mungbean grains for

Table 1. Trapping efficiency of two-in-one model trap for *C. maculatus* adults

Treatment	No. of adults trapped after# (Mean± SE)					
	1st month	2nd month	3rd month	4th month	5th month	6th month
Storage container with grains with one trap	53.17± 0.03^{b}	28.00± 0.01^{c}	24.00± 0.08^{c}	20.00± 0.09^{c}	17.00± 0.08^{c}	14.00± 0.16^{c}
Storage container with grains with one trap + sun drying of grains for 3 days at the end of each month	47.28± 0.05^{a}	12.37± 0.06^{b}	10.17± 0.08^{b}	9.30± 0.04^{b}	10.67± 0.02^{b}	11.23± 0.03^{b}
Storage container with grains with one trap + sun drying of grains for 5 days at the end of each month	50.17± 0.06^{a}	6.51± 0.06^{a}	4.00± 0.03^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
Untreated control	66.04± 0.12^{c}	110.80± 0.07^{d}	234.13± 0.16^{d}	281.38± 0.14^{d}	332.38± 0.13^{d}	436.25± 0.20^{d}
% weight loss after*# (Mean± SE)						
Storage container with grains with one trap	5.70± 0.18^{b}	4.00± 0.14^{c}	3.50± 0.23^{c}	3.28± 0.14^{c}	3.00± 0.12^{c}	2.50± 0.10^{c}
Storage container with grains with one trap + sun drying of grains for 3 days at the end of each month	5.13± 0.09^{a}	2.33± 0.13^{b}	1.85± 0.15^{b}	1.40± 0.10^{b}	1.67± 0.12^{b}	1.75± 0.12^{b}
Storage container with grains with one trap + sun drying of grains for 5 days at the end of each month	5.37± 0.19^{a}	1.25± 0.13^{a}	0.70± 0.14^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
Untreated control	6.55± 0.11^{c}	10.02± 0.31^{d}	17.00± 0.22^{d}	20.00± 0.38^{d}	24.40± 0.48^{d}	29.00± 0.41^{d}
% Grain damage*# (Mean± SE)						
Storage container with grains with one trap	8.90± 0.15^{b}	7.20± 0.10^{c}	6.22± 0.14^{c}	6.02± 0.11^{c}	5.83± 0.08^{c}	4.20± 0.14^{c}
Storage container with grains with one trap + sun drying of grains for 3 days at the end of each month	8.32± 0.08^{a}	4.80± 0.09^{b}	4.02± 0.10^{b}	3.50± 0.16^{b}	3.81± 0.10^{b}	4.00± 0.09^{b}
Storage container with grains with one trap + sun drying of grains for 5 days at the end of each month	8.40± 0.07^{a}	2.50± 0.14^{a}	1.00± 0.15^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
Untreated control	9.60± 0.07^{c}	17.21± 0.11^{d}	25.41± 0.13^{d}	29.33± 0.28^{d}	33.11± 0.19^{d}	38.55± 0.34^{d}

* Means within same column followed by same letter are not significantly different (Duncan's MRT, $P < 0.05$).
*1000 grain count basis

three days (8.32) (T_2) was significantly lowest and it was statistically at par with 8.40 observed in storage container with one trap and sun drying of mungbean grains followed for five days (T_3). After two months of storage, T_3 treatment was significantly better than all other treatments. In another studies, the number of insect catches in wheat bag stack increased with increase in number of traps used at each layer and minimum grain damage (1.30%) was observed (Hategikimana et al. 2013). Three traps/layer captured significantly higher number of insects as compared to one and two traps/layer. These workers concluded that among the monitoring tools, stack probe trap detected more infestation of major wheat storage insect-pests in the commodities as compared to spear sampling methods. At the end of fourth month, no further grain damage was noticed in treatment T_3 as this treatment had completely trapped the pulse beetles present in whole of the sample as evident in Table 1. Finally sixth months of storage gave, % grain damage varied of 0.00 in T_3 to 38.55 in untreated control. Results are also in agreement with Shadia and Aziz (2011) which stated that detection methods for stored grain insects helps in locating infestations for early diagnosis and to ascertain the success of fumigation or other control measures undertaken. Therefore, it can be concluded that use of two-in-one model trap and sun drying of mungbean grains for five days significantly performed better than all other treatments during six months storage of mungbean grains. The enormous quantitative and qualitative losses caused by stored grain insects are of serious concern to nutritional security throughout the world. Their timely detection is must to prevent heavy losses. The use of traps offers numerous advantages over the standard sampling procedure of collecting small volumes of grain and sifting or incubating them. Monitoring with these traps gives a quick sign of population growth. In addition to use of two-in-one model trap, sun drying of grains was found to be an effective approach for the management of pulse beetles as sun drying of grains helps to kill the immature stages of the insect. This practice can be easily adopted by farmers for timely detection, monitoring of pulse beetles.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTION

RK, DKS and KSS contributed equally in designing and performing the experiment and all of the authors contributed equally in data analysis and preparation of the manuscript.

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SENSILLA ON THE WINGS OF *EURYDEMA DOMINULUS* (SCOPOLI)

Joycy M Kharthangmaw, Sudipta Choudhury*, Khirod Sankar Das, K Chanreila L Nonglait and Cynthia B Marwein

Entomology Laboratory, Department of Zoology,
North Eastern Hill University, Shillong 793022, Meghalaya, India
*Email: sudiptapinku@gmail.com (corresponding author): ORCID ID-0000-0003-1426-5790

ABSTRACT

***Eurydema dominulus* (Scopoli), a phytophagous insect and a pest of many plants. The external morphology of the sensilla on the wings were observed using scanning electron microscope. Based on their morphological structure, different five types of sensilla were distinguished: sensilla trichoidea, sensilla chaetica, sensilla basiconica, sensilla campaniformia and sensilla ampullacea. These sensilla can be related to have mechanosensory, chemosensory and proprioceptive functions. The possible roles of these sensilla are also discussed.**

Key words: *Eurydema dominulus*, adult, Hemiptera, forewing, hindwing, dorsal, ventral, SEM, sensilla, morphology, measurements, distribution

Heteroptera (true bugs) represent over 40,000 described species with half-membranous forewings and sucking mouthparts (Weirauch and Schuh, 2011). Pentatomidae, one of the largest groups in the Pentatomomorpha, includes over 900 genera and 4,500 species (Kikuchi et al., 2011). Majority of the pentatomid species are phytophagous, and some of them are known as notorious pests of crop plants. *Eurydema dominulus* (Scopoli), is one of main insect pests of cruciferous crops. Regardless of its economic importance the sensory structure of this insect in different parts of the body have not been subjected to detailed study. Sensilla are sensory receivers with peculiar locations at the insect body being placed at antenna, maxillary palps, proboscis, tarsi etc. Sensilla exist in several forms (Asdelkrim and Mehlhorn, 2006). In case of insects, locating a host plant is crucial to find suitable oviposition sites and to fulfil its nutritional requirements (Quicke et al., 1995). In most herbivorous insects, survival of offspring mostly depends on the selection of suitable host plant (Renwick and Chew, 1994). Plant chemicals influence host plant location and acceptance of suitable plants for feeding and oviposition. Detection of these chemical stimuli is accomplished by an array of sensory capabilities, the gustatory and olfactory sensilla present on antennae and other parts of insect body (Ananthakrishnan, 1992)

Scanning Electron Microscopy (SEM) has become an indispensable and inevitable technique for studying the detailed morphology of cuticular structures (Dey, 1995). SEM for studying the distribution pattern, directional function, structural features of antennal socket, specialization in position, the nature of association of the sensilla base with the body cuticle and finer detail of the surface of sensilla has been studied by Dey (1995), Dey and Biswas (1996). Insect wings have many sensilla near wing bases and along wing veins and margins which are responsible for sensing as well as for generating airflow (Pringle, 1957; Albert et al., 1976; Palka et al., 1979; Cole and Palka, 1982; McIver, 1985; Yack et al., 2000; Yoshida et al., 2001). The articulation between the wing and body plays an important role in insect wing movement (Snodgrass, 1935), sensilla near the wing bases of several insects have been studied in terms of wing proprioception: neuronal information from campaniform sensilla near the wing bases of locusts (Gettrup, 1966). Sensilla not located near wing bases may also play a complementary role in wing movement regulation, but detailed studies of these roles have not been performed, therefore the present paper gives a description of the external morphology, and distribution of the sensilla that are present on both the fore and hind wings (dorsal and ventral) of *Eurydema dominulus* (Scopoli). The possible role of these sensilla in relation to the insect's behaviour is discussed in relation to the current available literature.

MATERIALS AND METHODS

The insect samples were collected from Umbir village, Ribhoi district (Meghalaya) by hand picking method. The fore wings and hind wings were carefully excised from the insect by using fine forceps. For

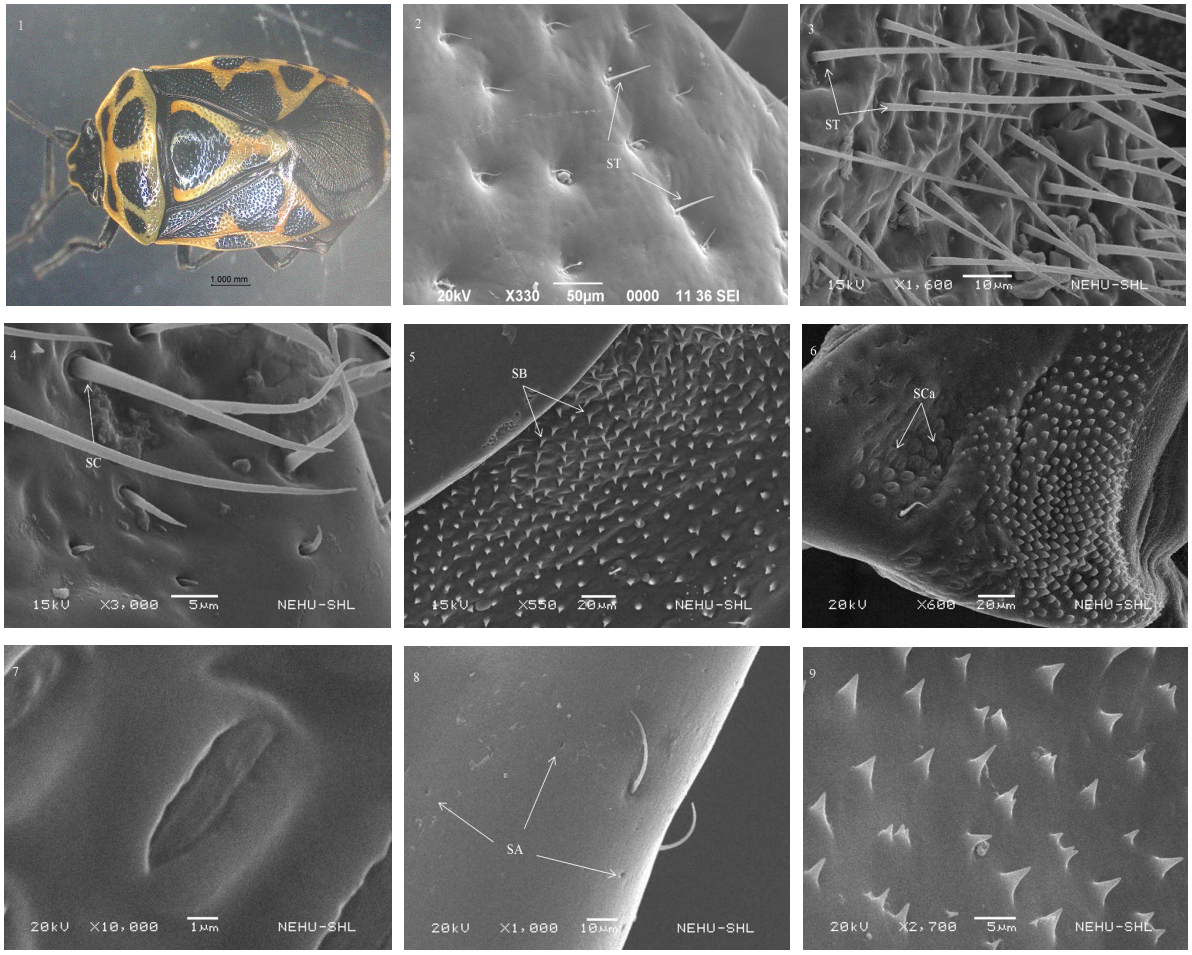
scannig electron microscopy, samples were prepared following the method of Dey et al.,1989. Samples were fixed in 2.5% Glutaraldehyde for 24 hr at 4°C and washed 3 times in 0.1 M Sodium Cacodylate buffer for 15 min in each change at 4°C. Then the samples were dehydrated in ascending grades of acetone (30%, 50%, 70%, 80%, 90%, 95% and 100%), keeping in each grade for 15 min for 2 times. After dehydration, samples were dried in Tetramethylsiline for 5-10 min for two times and air dried at room temperature. Then the specimens were mounted on aluminium stubs with the help of double adhesive tapes followed by a 35 mm gold coating (in sputter) and viewed under Scanning electron microscope (Jeol- JSM 6390). The sensilla on the wings of *Eurydema dominulus* were classified following all the available literature and the measurements of the sensilla were done from the SEM micrographs using image J software (latest version).

RESULTS AND DISCUSSION

Based on their morphology, distribution and cuticular attachment, five types of sensilla were identified. They were classified as: sensilla trichoidea (ST), sensilla chaetica (SC), sensilla basiconica (SB), sensilla campaniformia (SCa) and sensilla ampullacea (SA). No sensilla were observed on the membranous portion of the fore wing. Sensilla trichoidea (ST) are porous or aporous, ribbed or smooth, with a slightly rounded or sharp tip and flexible or inflexible sockets. These sensilla were observed in the fore wings (dorsal) in between the radius, media and corium of the wings (Fig. 1-3; Table 1). The structural features of the sensilla indicated that they function as mechanoreceptors (Thurm, 1964, 1965; Gaffal and Hansen, 1972; Altner et al., 1983; Gnatzy and Tautz, 1980; French and Sanders, 1981). Apart from its mechanosensory role, sensilla trichoidea also appears to play a chemosensory function.

Sensilla chaetica (SC) are stiff hair like structures that are long and straight with a blunt and tapering tip and are thicker than sensilla trichoidea. All of their stems are ribbed and arise from a cuticle with a flexible socket (Fig. 4; Table 1). These sensilla were observed on the fore wings and hind wings (dorsal side). Sensilla chaetica may play some mechanical role, since they have been shown to be non-innervated (Schmidt and Smith, 1985). Variety of insects representing different orders have been shown to be contact chemo-sensilla (Kaestner, 1972; Wigglesworth, 1972; Horn, 1982). In many cases these receptors also respond to the presence of dissolved carbohydrates (Anderson, 1932) and are involved in the feeding responses of the insects. In *Eurydema dominulus*, the distribution pattern of sensilla Chaetica suggests that they are in contact with the substrate when the insect moves or stands. The porous nature of the sensilla as revealed by magnified SEM micrographs suggests their chemoreceptive role (Faucheux, 1991). In this context, it should be mentioned here that the contact-chemoreceptive function of sensilla chaetica has been previously demonstrated in some lepidopterans (Anderson and Hallberg, 1990).

Sensilla basiconica (SB) are cones that arise from flexible or inflexible sockets. Their surface may be porous or aporous. The stem of the sensillum is thick at the base and tapers upwards (Fig. 5; Table 1). Sensilla basiconica were observed on the dorsal side of the forewing and hindwing. Silva et al. (2010) suggested that this type of sensillum may be related to finding food or suitable habitats. Someauthorshaveassignedathermo- or chemoreception function to these sensilla (Chapman 1982, Zacharuk 1985). Sensilla campaniformia (SCa) are flat, oval shaped discs. They are sparsely distributed on anterior region of both the fore and hind wings and have flexible sockets (Fig. 6,7; Table 1). Campaniform sensilla were observed at the wing base on the dorsal side of forewing and hindwing. Sensilla campaniformia are also found on various parts of the insect's body such as wings, ovipositor, antenna, mouth parts and legs (Pringle, 1957). They result from cuticular stress due to compression and stretching of the surrounding cuticle. The campaniform sensilla has directional sensitivity which has been described by Pringle (1968) and Chapman (1965). The position and orientation of the sensilla in the joint or anterior region of the wings suggest that they monitor strains that develop during dorsal and ventral bending. In addition, campaniform sensilla could also provide information about the muscular force required to maintain a fixed relation between body parts despite changes in the insect's position relative to gravity (Horn, 1985). Sensilla ampullacea (SA) are pegs set at the bottom of a tube internally but appear as small round openings on the cuticular surface externally. Peg set at the bottom of long tube. Fore wings (dorsal& ventral) and hind wing (dorsal) are sparsely distributed by this type of sensilla (Fig. 8, 9; Table 1). However, it is known that pit organs or sensilla ampullacea function as carbon dioxide, temperature and humidity receptors in some Hymenoptera (Lacher, 1964). Similar roles can be assigned to the cuticular pits in the wings.



Figs. 1-9. 1. Adult of *Eurydema dominulus* (Scopoli), 2. Sensilla trichoidea (ST) on the non-membranous parts of fore wing, 3. Sensilla trichoidea on the base of the forewing, 4. Sensilla chaetica (SC) on the dorsal side of the forewing, 5. Sensilla basiconica (SB) on the dorsal side of hindwing, 6. Sensilla campaniformia (SCa) on the base of the dorsal side of fore wing, 7. SCa at higher magnification, 8. Sensilla ampullacea on the dorsal side of fore wing, 9. Spines-like structure (microtrichia) on the dorsal side of fore wing.

Table 1. Sensilla types, location, width and possible function- wings of *E. dominulus*

S. No.	Sensilla	Location	Length (µm)	Width (µm) Basal/ Distal	Possible function
I	Sensiila trichoidea	Forewings (dorsal & ventral)	25–35	1–3 /0.5–0.8	Mechanoreception and chemoreception
II	Sensilla chaetica	Forewings (dorsal & ventral)	28.8–32.2	2.3–3.6/0.4–2.3	Mechanoreception
III	Sensilla basiconica	Fore & hind wings (dorsal & ventral)	2.3–2.5	1.5–1.8 /0.2–0.4	Olfaction and Chemoreception
IV	Sensilla campaniformia	Fore & hind wings (dorsal)		5.4–6.3 (diameter)	Directional sensitivity and proprioceptive
V	Sensilla ampulacea	Fore wings (dorsal)		–	Thermo-hygro receptive

The sculpturing pattern of the cuticle in the form of some plates, spines like (microtrichia, Fig. 9) and folds observed in wings may serve to reduce friction between the cuticle and the surface of the plant part. This pattern may also facilitate accumulation of any secretion from the insect's body. It is believed that these cuticular structures are formed at locations on the insect's body where maximum forces of friction are generated (Amrine and Lewis, 1978). In this present study, five types of sensilla were identified, measured and characterized for the first time both the morphology and distribution of sensilla located on the wings of *Eurydema dominulus*. Thus, this study allows us to better understand the possible role and functions of each sensilla. Further studies using transmission electron microscopy coupled with electrophysiological recordings are likely to confirm the specific functions of different sensilla identified in this study.

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POPULATION DYNAMICS OF SIX SPECIES OF *CULEX PIPIENS* L. GROUP (DIPTERA: CULICIDAE) FROM CHANDIGARH#

Anupika Sood, Ranjana Rani[1], Sukhbir Kaur and Sagandeep Kaur[1]*

Department of Zoology, Panjab University, Chandigarh 160014, India
[1]Department of Zoology, DAV College Sector-10, Chandigarh 160011, India
*Email: mailtoshagan@yahoo.co.in (corresponding author): ORCID ID 0000-0001-7165-7219

ABSTRACT

During the present study, detailed mosquito surveys were carried out from various habitats of developed areas, gardens, slums and surrounding villages of Chandigarh from June 2017 to November 2019 to explore the mosquito fauna. Six species of *Culex pipiens* L group were recorded with maximum distribution of *Culex quinquefasciatus* Say (89.61%) followed by *Culex univittatus* Theobald (6.39%) while, rest of the species *viz; Culex vagans* Wiedemann, *Cx. hutchinsoni* Barraud, *Cx. theileri* Theobald and *Cx. fuscocephala* Theobald collectively formed only 3.99% in almost in all habitats. The population dynamics was analyzed from regular surveys to assess the relationship between abundance of six vector species of *Cx. pipiens* group with meteorological variables. The data revealed varied abundance of species of *Cx. pipiens* group in different seasons. Survey of literature revealed that no data is available on the species of *Cx. pipiens* group in the region with particular reference to weather parameters. Hence, the present study on the seasonal abundance and population dynamics of *Culex pipiens* group in Chandigarh and its surroundings has been carried out.

Key words: *Culex pipiens*, Chandigarh, species diversity, vectors, weather parameter, abundance, population dynamics, correlation coefficients, distribution, seasonal abundance

The earlier taxonomic workers differentiated the species of *Culex* into groups, subgroups and complexes. Edwards (1932) categorized the species of *Culex* into two groups namely the *pipiens* group and *sitiens* group in first catalogue of mosquitoes of the world. Later on Sirivanakarn (1976) arranged the species under *Culex pipiens* group into four subgroups i.e., *pipiens* subgroup, *trifilatus* subgroup, *theileri* subgroup and *univittatus* subgroup and placed the closely related species into these subgroups accordingly. The present studies are subjected to seasonal dynamics of six species i.e., *Culex quinquefasciatus* Say (*pipiens* subgroup), *Cx. vagans* Wiedemann and *Cx. hutchinsoni* Barraud (*trifilatus* subgroup), *Cx. theileri* Theobald (*theileri* subgroup), *Cx. univittatus* Theobald and *Cx. fuscocephala* Theobald (*univittatus* subgroup). The first catalogue of Indian mosquitoes given by Tyagi et al. (2015) also mentioned these six species of *Cx. pipiens* group out of 404 species collected from various geographical areas of India. The species under *Culex pipiens* group especially *Cx. quinquefasciatus* and *Cx. fuscocephala* are particularly important in public health in India due to their potential to transmit various diseases like Japanese encephalitis, periodic filariasis, West Nile Virus, St. Louis encephalitis, Rift Valley fever viruses, and Sindbis virus (National Center for Vector Borne Diseases Control, 2022; Amara et al. 2016). However, *Cx. univittatus* and *Cx. theileri* are responsible for carrying West Nile virus, Sindbis virus, bancrofti fever, Bagaza virus, Western Equine encephalitis virus and other viral pathogens globally (Walter Reed Biosystematics Unit 2022). It is worthwhile to mention that the environmental factors such as temperature, rainfall and relative humidity play a very significant role in the transmission of various pathogens. Hence, keeping in view the medical importance of species of *Cx. pipiens* group, the present study explored the species distribution and seasonal prevalence in Chandigarh and its adjoining areas.

MATERIALS AND METHODS

The survey cum collection of mosquitoes was carried out during morning as well as evening hours from June 2017 to November 2019 from different habitats in and around Chandigarh (30° 44' N, 76° 46' E). Hand collection method was followed in which hand sweep nets and oral aspirators were used. The collected samples were brought to laboratory, pinned and preserved in the insect storage boxes. The standard taxonomic keys of Barraud 1934, Sirivanakaran 1976

#Table 1-3 are supplementary and are available only in online version

and Reuben 1994 were used to identify mosquitoes. Further, slides of male genitalia were prepared using the method given by Siverly and Shroyer (1974) and photographed. Meteorological data was collected for the period June 2017 to November 2019 from Meteorology Department, Sector 39, Chandigarh, India. The standard parameters like temperature (maximum and minimum), rainfall and relative humidity were taken. The data was analyzed to know relationship of these weather factors with population density, using Pearson correlation coefficient. Regression analysis including p value and correlation coefficient (r) was performed to evaluate the statistical significance ($p< 0.05$) using SPSS® 16.0.

RESULTS AND DISCUSSION

During June 2017 to November 2019, a total of 2802 mosquitoes belonging to six species of *Culex pipiens* group namely *Cx. quinquefasciatus, Cx. univittatus, Cx. fuscocephala*, *Cx. vagans* and *Cx. hutchinsoni* and *Cx. theileri* were collected and studied. The results revealed that *Cx. quinquefasciatus* and *Cx. univittatus* were the most prevalent species. The density of *Cx. quinquefasciatus* was maximum in garden belts (37.6%) followed by developed urban areas (26.1%), villages (25%) and slum areas (10%) whereas, *Cx. univittatus* exhibited its highest density in villages (39.1%) followed by developed areas (25.6%), garden belts (20.1%) and slums (15%). The *Cx. vagans* was also prevalent mostly in villages (40.4%) followed by developed areas (33.3%), slums (21.4%) and garden belts (4.76%). *Cx. hutchinsoni* too was highest in villages (43.3%) followed by developed areas (20%), garden areas (20%) and then slums (16.6%). The *Cx. theileri* also showed its maximum density in villages (42.3%) followed by developed areas (23.07%), garden belts (23.07%) and slums (11.53%). *Cx. fuscocephala* showed its high abundance in villages (62.5%) followed by garden belts (18.75%), slums (12.5%) and urban areas (6.26%) (Fig. 1).

The seasonal incidence of both *Cx. quinquefasciatus* and *Cx. univittatus* were observed with peak in July to August, when maximum and minimum temperature was 33.39°C and 26.69°C, relative humidity 68% and rainfall 8.31mm. *Cx. vagans* showed its appearance in June, when temperature range was 27-35.2°C and reached at its highest peak in July to August, when maximum and minimum temperature was 33.41°C and 26.69°C, relative humidity was 68.05% with 8.31mm of rainfall and remained very low during September to November. *Cx. hutchinsoni* too started appearing with its highest peak in June to July when maximum and minimum temperature was 35.08°C and 26.88°C, relative humidity 54.4% and rainfall (5.61mm) and its abundance declined in August to September, but it again appeared in October and November. *Cx. theileri* was found with very low density in June to August and reached to highest peak in October and November when maximum and minimum temperature was 29.11°C and 18.32°C, relative humidity was 52.92% with 0.81mm rainfall. *Cx. fuscocephala* appeared in July with peak

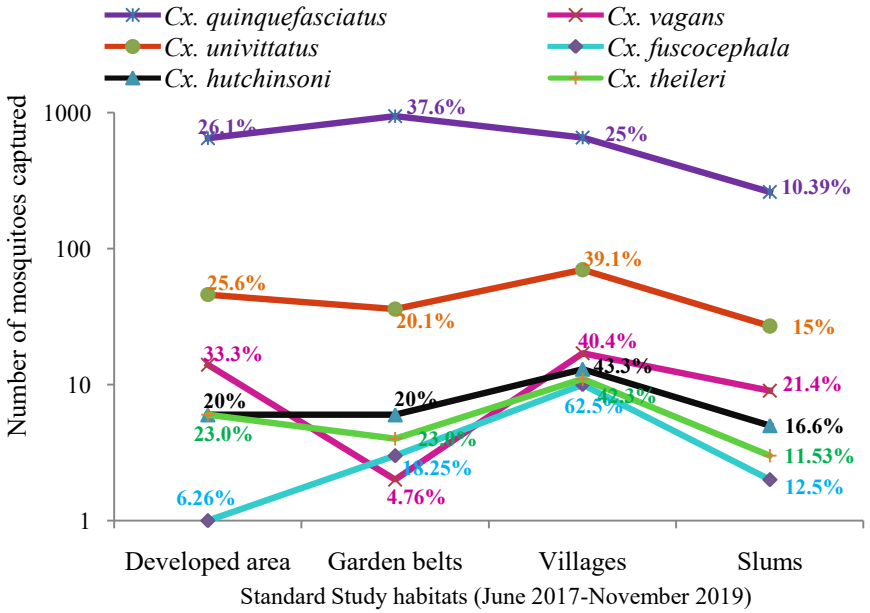

Fig. 1. Diversity of species of *Culex pipiens* group in habitats of Chandigarh (2017-2019)

in October, when maximum and minimum temperature was 31.83°C and 21.38°C, relative humidity 47.94% with 0.10 mm rainfall. Its density was observed very low in August with negligible appearance in June and September. The pooled data revealed that all the species were diversified in different habitats and its seasonal abundance in an area firstly appeared in June, gradually increased and reached to peak during July to August while, declined gradually in September to November (Fig. 2).

The coefficient of determination exhibited a negative correlation of *Cx. vagans* with relative humidity (r=-0.86) in 2017. In 2018, *Cx. hutchinsoni* was strongly positive correlated with relative humidity (r=0.81). While in 2019, it was found to be negatively correlated with maximum temperature (r=-0.79) and minimum temperature (r=-0.83). The incidence of *Cx. fuscocephala* was strongly negative correlated with minimum temperature (r=-0.84). *Cx. quinquefasciatus* and *Cx. vagans* found to be positively correlated with rainfall (r=0.9, r=0.82) (Fig. 3). However, no significant correlations were observed among species with weather parameters in remaining years of study. Hence, the present data revealed that the relative changes in population density of collected mosquito species is complex and can be affected by prevailing weather parameters.

Various other workers who worked on mosquito abundance in relation to climate factors also observed a similar pattern. Bhat and Kulkarni (1983), recorded *Cx. quinquefasciatus* and *Cx. fuscocephala* in October-November, while *Cx. theileri* and *Cx. vagans* in August-October from Jammu and Kashmir. Sharma (1997) and Fakoorziba and Vijayan (2006) found peak density of *Cx. quinquefasciatus* in July and August in Gurgaon (Haryana) and in Mysore (Karnataka) respectively. Kanojia and Geevarghesh (2005) observed first peak density of *Cx. quinquefasciatus* in March–April followed by September-October in Gorakhpur. However, in Saudi Arabia, Alahmed (2012) observed peak density of *Cx. quinquefasciatus* and *Cx. univittatus* in June, when temperature reached 36°C, its abundance started declining with decrease in temperature (15°C). It further increased in rainfalls during January to March. It could be due to increase in breeding sites in these months. Roiz et al. (2014) found abundance of *Cx. theileri* in April to August in Placibo, Mediterranean wetlands. They also revealed negative correlation of *Cx. theileri* with relative humidity. In Agra, Shad and Andrew (2016) during three years study, noticed peak larval density of *Cx. quinquefasciatus* from July to September during first year, August to October during the second year and August to September during the third year. Bashar et al. (2016), observed *Cx. hutchinsoni* along with dominant species *Cx. quinquefasciatus* during May to June months of Dhaka, Bangladesh in semi-urban areas. In Bareilly, Pantawane et al. (2017) too noticed two peak density months of *Cx. quinquefaciatus*, first in Feburary to March when temperature range was 12-29°C and second peak in September to October. They revealed that mosquito population increased with overall increase in maximum temperature (21-39°C), rainfall and relative humidity (40-60%). The *Cx. fuscocephala* was also seen highest in monsoon season i.e. July to September and in post-monsoon seasons. Mohamed et al. (2020), studied seasonal variation of both *Cx. quinquefasciatus* and *Cx. univittatus* from Khartoum state of Sudan, Saudi Arabia and found high peak incidence during pre-rainy season (April–July). Manzoor et al. (2020), studied *Cx. vagans* along with *Cx. quinquefasciatus,* and observed its maximum prevalence during September 2014 to August 2015 in Lahore, Pakistan in different breeding habitats.

Present study revealed that collected species marked their peak relative abundance majorly in garden belts, followed by villages, developed urban areas and then slums. It might be due to the availability of suitable breeding sites like vegetation, bamboo trees, ditches, flower pots, catch basins, water logs and puddles with diverse water quality in gardens. In villages, irrigated agricultural lands, cattle sheds, small water bodies with algal blooms provide more breeding habitats than in developed urban areas with semi water logs conditions during rainy seasons. The slums which contributed one fourth of its abundance could be due to the reason that majority of the species of *pipiens* group breeds in fresh water, whereas the polluted water bodies, garbage storage lands, waste muddy water bodies in slums provided less breeding sites. Hence, it can be inferred that out of all climatic parameters, the rainfall pattern has a strong influence on the breeding of *Cx. quinquefasciatus* and *Cx. vagans* whereas, population density of *Cx. fuscocephala* and *Cx. hutchinsoni* was strongly associated with temperature changes. The relative humidity influenced incidences of *Cx. vagans* and *Cx. hutchinsoni* in urban and rural areas of Chandigarh. The influence of climatic variables on the seasonal dynamics of vector species of *pipiens* group can be useful for predicting future transmission pattern and to strengthen vector control strategies with

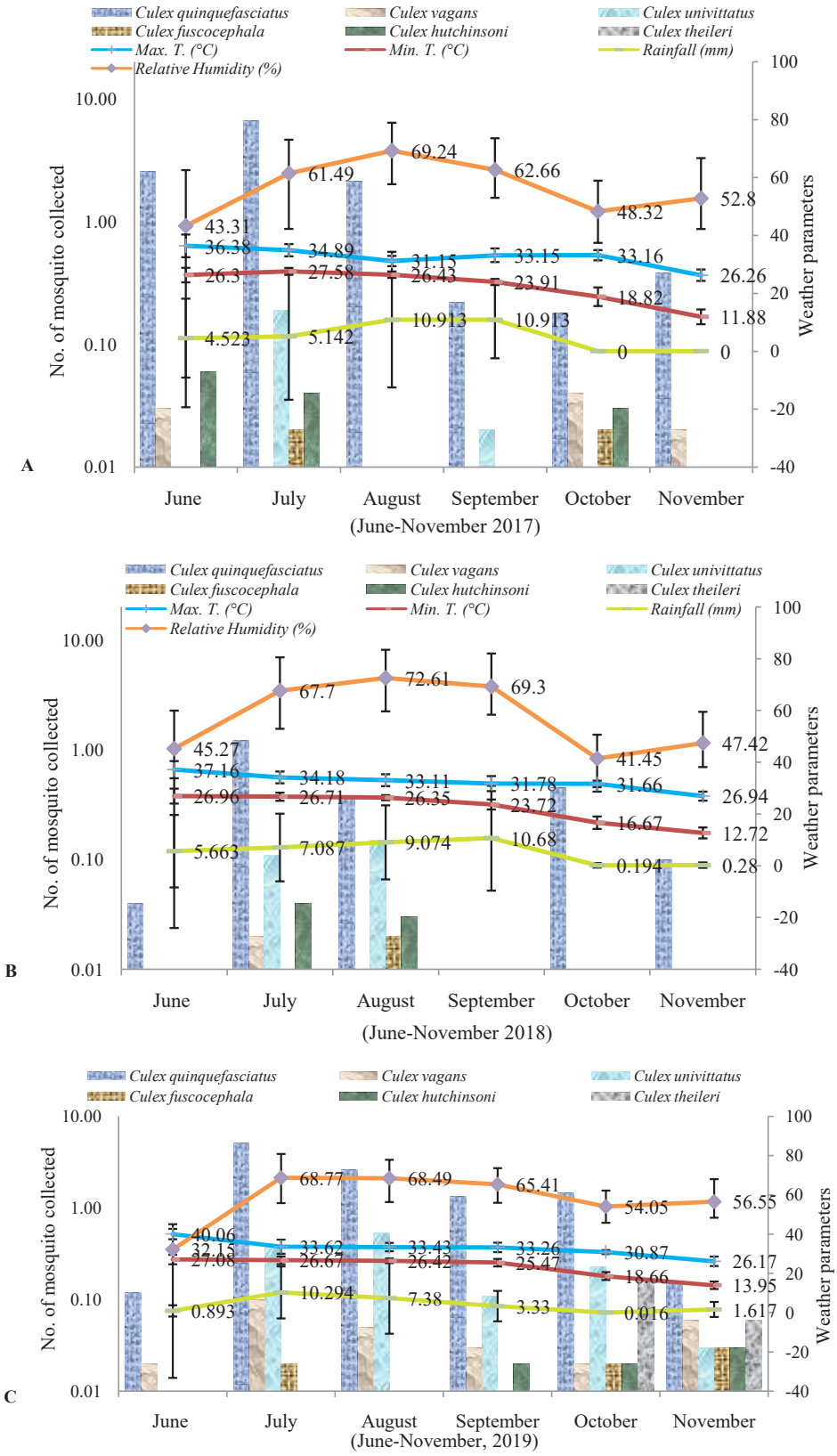

Fig. 2. Seasonal incidence of *Culex pipiens* group (pooled data)- Chandigarh (2017-2019)

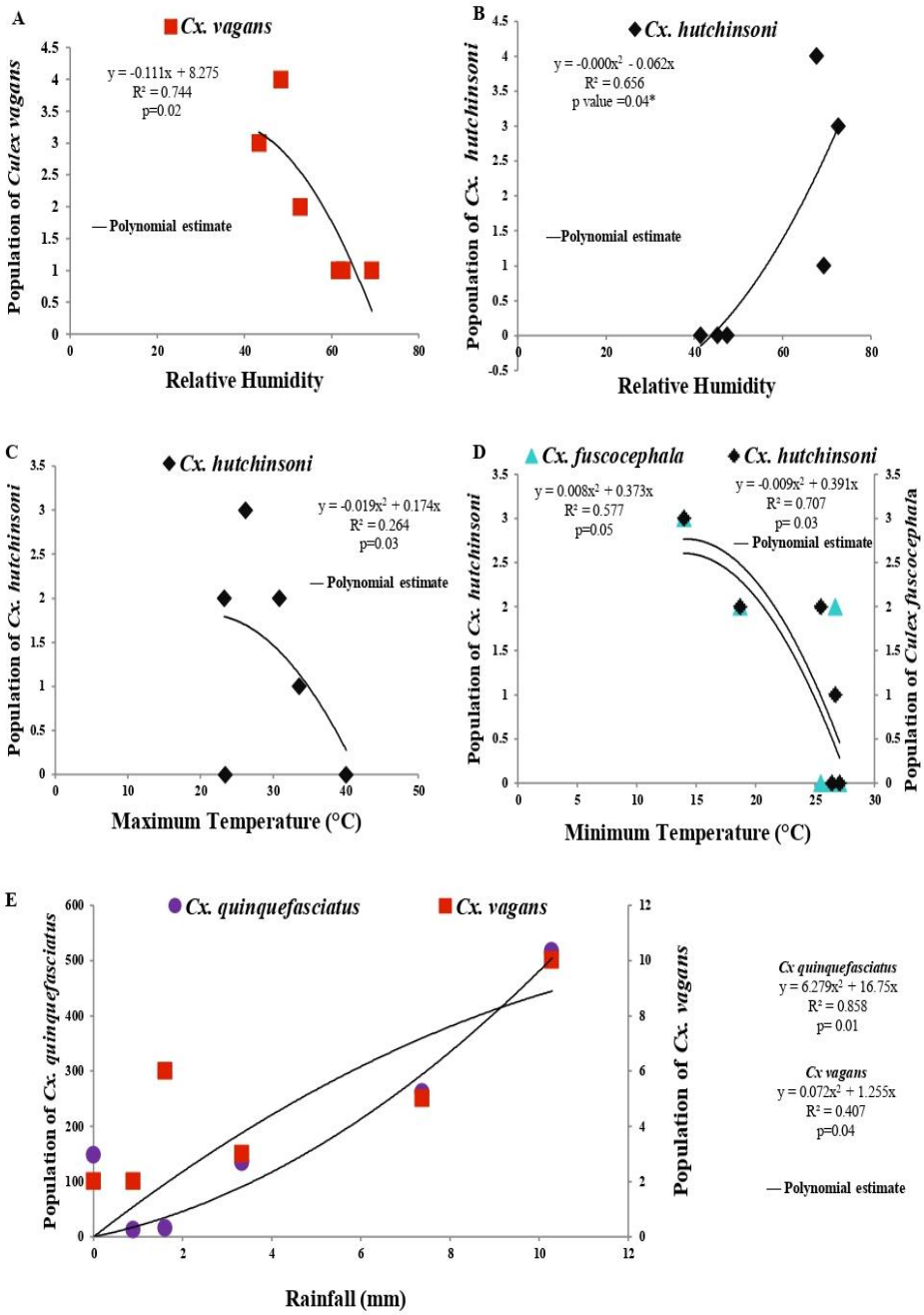

Fig. 3. Population dynamics of *Culex pipiens* group (Chandigarh- 2017 to 2019)

surveillance programme in and around Chandigarh proximities in an effective manner.

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AUTHOR CONTRIBUTION STATEMENT

AS and RR devised and designed research. AS and RR conducted surveys and experiments. SK suggested

statistical analysis. AS, RR, and SK analyzed data and wrote the manuscript. All of the authors read and approved the manuscript.

CONFLICT OF INTEREST

There are no conflicts of Interest.

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*Table 1-3 are supplementary and are available only in online version

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PREDICTING AVIAN DIVERSITY WITH GEOSPATIAL TECHNOLOGIES

Tejdeep Kaur Kler*, Hasneet Kaur Hora, Anil Sood[1] and Prabha Kumari[2]

Department of Zoology, Punjab Agricultural University, Ferozepur Road, Ludhiana 141004, Punjab, India
[1]Agroecosystems and Crop Modelling Division, Punjab Remote Sensing Centre, Ludhiana 141004, Punjab, India
[2]Punjab Remote Sensing Centre, Ludhiana 141004, Punjab, India
*Email: tejdeepkler@pau.edu (corresponding author)

ABSTRACT

Rapid urbanization accompanied by land use changes is affecting biodiversity worldwide specifically avian diversity. In the present study, the interactions of avian diversity with vegetation area were evaluated using geospatial technologies at different locations of Ludhiana city from April 2019 to March 2020. Integration of observations was done to analyze the avian diversity in relation to habitat components. Bird surveys conducted have recorded 46 bird species. There were 44 tree and 14 shrub species recorded, with vegetation and buildup area being the key determinants of diversity. Urban locations with balanced buildup area and vegetation were observed to support bird species diversity.

Key words: Avifauna, bird orders, Bray-Curtis dissimilarity index, buildup, correlation, feeding guilds, habitat, remote sensing, urbanization, vegetation

Bird diversity and its composition are affected by increase in urbanization at global level (Leveau et al., 2017). A city's biodiversity is linked to the habitat components and vegetation heterogeneity, which is influenced by the anthropogenic activities (Beninde et al., 2015). Urbanization expanding at global rate has affected bird diversity and composition drastically (Leveau et al., 2017). With considerable effect on biodiversity driven by increasing urbanization, there is a need to study the characteristics of urban green spaces which might be preserved or manipulated to improve biodiversity conservation outcomes (Lepczyk et al., 2017). Tree species assist in urban avifaunal maintenance and they provide food and water to birds and thus promote biodiversity of the city (Silva et al., 2020). Altaf et al. (2018) pointed about fragmentation of natural bionetwork of environment specifically the habitat preferred by avifaunal species in urbanized landscape because of removal of natural vegetation. Satellite based data on regionalization are more amendable to change as new remotely sensed data can be readily added to allow the regionalization to adapt to changes in terrestrial conditions either due to climate or land use change as this data captures fine-scale spatial patterns (Coops et al., 2018, Rocchini et al., 2018). A new approach for green space analysis in an urbanized environment has been presented in the form of a tool for mapping perceived quality of remote sensing (Stessens et al., 2020). In India, urbanization increased from 27.81% in 2001 to 31.16% in 2011 with an increase of 91 million in urban population leading to sudden rise in population growth rate and land use changes (Chandramouli 2016). Singh and Kalota (2019) deduced 8% growth rate of buildup area in Ludhiana by combining the remotely sensed data and geographic information system data in 2015. Thus, keeping this in view, geospatial technologies were utilized to analyze avian diversity in relation to vegetation and other habitat components in Ludhiana, Punjab.

MATERIALS AND METHODS

The study was carried out in Ludhiana city, Punjab (30.9010°N,75.8573°E) between April 2019 to March 2020. Ludhiana features a humid subtropical climate with roughly 890 mm of precipitation annually. Birds were studied along four selected locations taken as location 1, 2, 3 and 4. Rajguru Nagar (location 1) is a well-developed housing structure. Native and introduced vegetation observed. Agar Nagar (location 2) has maximum buildup area and least vegetation pertaining to highly urbanized residential area including high human disturbances. Dugri Estate (location 3) had modern housing structures with mature trees and newly planted saplings were observed. Residential houses lacking structural diversity and characterized by diversity of tree types both as rows along lanes and

fruit trees dotting backyards were observed at Punjab Agricultural University Campus (PAU) (location 4).Two transects were taken per location and their data on bird species abundance and richness was pooled. The studied locations were categorized by buildup area, vegetation, parks, empty plots and road. Locations were visited four times in a month and line transect method was followed taking 1 km transect to record bird species using binoculars (7X50) (Verner 1985). The identification of birds was based on the keys given in Ali (1996). Identification of tree species at studied locations was done according to Sahni (1998). Birds species recorded were assigned to six broad feeding guilds: frugivore (F), carnivore (C), granivore (G), omnivore (O), insectivore (I), nectarivore (N) as classified by Shanahan et al. (2011). Remote sensing data was obtained from the Punjab Remote Sensing Centre, Ludhiana. It was processed through ArcGIS (Arc Map 10.4) to calculate various habitat components of the four selected locations. Each selected location was classified into buildup area, vegetation, parks, fellow land, road cover and divided into polygons and area of each cover type was calculated. To understand location wise bird diversity, comparison of species along with their feeding guilds was carried out. Pearson's Correlation analysis was used to evaluate the relationship between feeding guilds and their habitat components; also, between species richness and two set of variables (i.e. vegetation and buildup area). The dissimilarity across the studied locations using bird species richness was depicted by Bray-Curtis dissimilarity index (Bray and Curtis, 1957).

RESULTS AND DISCUSSION

A total of 46 bird species were recorded belonging to 29 families and 12 orders from April 2019 to March 2020 (Table 1). Columbiformes had five species namely eurasian collard dove, laughing dove, spotted dove, rock pigeon and yellow-legged green pigeon. Two raptor species black kite and shikra belonging to Accipitriformes were observed. Spotted owlet belonging to Strigiformes was observed only at location 3 (Table 1). Indian peafowl was observed only at location 3 and black francolin was observed only at location 4, both belonging to order Galliformes. Overall bird composition revealed that rock pigeon (28.13%) was the most abundant commensal species followed by common myna (14.92%) and house crow (10.60%). The structural features like road cover, vegetation cover, buildup, fellow land and park of all the studied locations were noted and their area was calculated. Road cover was maximum at location 3 (32.56%), followed by location 1 (27.75%), location 2 (18.48%) and location 4 (8.96%). Vegetation cover came out to be maximum at location 4 (61.92%), then location 3 (19.42%) followed by location 1 (15.06%) and location 2 (5.12%). Buildup was maximum at location 2 (68.59%) followed by location 1 (46.01%), location 3 (38.68%) and location 4 (21.20%). Fellow land was found covering maximum area at location 4 (5.88%), then location 1 (4.74%) followed by location 3(2.21%) and location 2 (2.14%). Parks covered most area at location 3 (7.13%) followed by location 1 (6.43%), location 2 (5.68%) and location 4 (2.05%). A total of 44 tree and 14 shrub species were recorded of which 30 tree species were native, one naturalized (gulmohar) and rest 13 were introduced. Among 14 shrub species, 6 species were native whereas one species is naturalized (earleaf acacia) and 8 species were introduced; 29 species were evergreen and 15 species were deciduous. 32 tree species out of 44 tree species were present at location 4 indicating rich vegetation heterogeneity and represented by maximum vegetation area (Table 2). From all the tree species recorded: banyan, peepal, banyan, chinaberry, mango and lemon were observed to be utilized by 25 bird species. Bray-Curtis dissimilarity index for bird orders revealed that maximum difference was observed between locations 1 and 3 followed by difference between locations 3 and 4, locations 2 and 4, locations 1 and 2, locations 2 and 3 and locations 1 and 4. Eight bird species were exclusive to location 4 and were not observed at other three locations. Rock pigeon and common myna were found in greater abundance at locations 1, 2 and 3 as these bird species seemed to be more adapted to buildup areas for their nesting, roosting, feeding and other requirements as was evident at location 2. Buildup area had been found to have a higher influence on the bird species richness. The correlation analysis between vegetation, buildup area and species richness showed a positive correlation of bird species richness with vegetation whereas it was vice versa for buildup area. Bird species observed belonged to various feeding guilds; frugivores (5), carnivores (4), omnivores (15), insectivores (14), nectarivores (1) and granivores (7). Correlation between feeding guilds and structural features revealed that carnivores were negatively correlated to vegetation as well as buildup. Frugivores and omnivorous were positively correlated to vegetation but negatively correlated to buildup (Table 3) as both frugivores and omnivoresseem to depend on vegetative cover for their feeding activities whereas granivores, insectivores and nectarivores were negatively correlated to vegetation but positively correlated to buildup. This study reflected that bird

Table 1. Bird diversity in Ludhiana, Punjab (April 2019 to March 2020)

S. No.	Common name	Scientific name	L1	L2	L3	L4	Trophic Group	Migratory Status
1	Asiankoel	*Eudynamys scolopaceus*	-	-	-	✓	F	R
2	Asian pied starling	*Gracupica contra*	-	-	✓	-	O	R
3	Bank myna	*Acridotheres ginginianus*	✓	-	✓	-	O	R
4	Blackdrongo	*Dicrurus macrocercus*	✓	✓	✓	✓	I	R
5	Black francolin	*Francolinus francolinus*	-	-	-	✓	O	R
6	Black kite	*Milvus migrans*	✓	✓	✓	✓	C	R
7	Black redstart	*Phoenicurus ochruros*	-	-	✓	-	O	WM
8	Brahminystarling	*Sturnia pagodarum*	-	✓	-	✓	O	R
9	Brown rock chat	*Oenanthe fusca*	-	✓	✓	-	I	R
10	Brown-headed barbet	*Psilopogon zeylanicus*	-	✓	-	✓	F	R
11	Cattle egret	*Bubulcus ibis*	✓	✓	✓	✓	O	R
12	Common chiffchaff	*Phylloscopus collybita*	✓	-	-	-	I	WM
13	Common hawk cuckoo	*Hierococcyx varius*	-	-	-	✓	I	R
14	Common hoopoe	*Upup aepops*	-	-	✓	✓	I	R
15	Common myna	*Acrido therestristis*	✓	✓	✓	✓	O	R
16	Common starling	*Sturnus vulgaris*	-	-	✓	-	O	WM
17	Common tailorbird	*Orthotomus sutorius*	✓	✓	✓	✓	I	R
18	Eurasian collared dove	*Streptopelia decaocto*	✓	✓	✓	✓	G	R
19	Greater coucal	*Centropus sinensis*	-	-	-	✓	O	R
20	Green bee-eater	*Merops orientalis*	-	-	✓	-	I	R
21	House crow	*Corvus splendens*	✓	✓	✓	✓	O	R
22	House sparrow	*Passer domesticus*	-	✓	-	✓	O	R
23	Indian grey hornbill	*Ocyceros birostris*	-	-	✓	✓	F	R
24	Indian peafowl	*Pavo cristatus*	-	-	✓	-	O	R
25	Indian robin	*Saxicoloides fulicatus*	✓	✓	✓	✓	I	R
26	Indian silverbill	*Euodice malabarica*	✓	✓	-	-	G	R
27	Jungle babbler	*Turdoides striata*	✓	✓	✓	✓	I	R
28	Large grey babbler	*Argyam alcolmi*	-	-	✓	-	I	R
29	Laughing dove	*Streptopelia senegalensis*	✓	✓	✓	✓	G	R
30	Lesser golden-backed woodpecker	*Dinopium benghalense*	-	-	-	✓	O	R
31	Oriental magpie robin	*Copsychus saularis*	-	✓	-	✓	I	R
32	Pied bushchat	*Saxicola caprata*	-	-	✓	-	I	R
33	Purple sunbird	*Cinnyrisasiaticus*	✓	✓	✓	✓	N	SM
34	Red-vented bulbul	*Pycnonotus cafer*	✓	✓	✓	✓	O	R
35	Red-wattledlapwing	*Vanellus indicus*	✓	✓	✓	✓	I	R
36	Rock pigeon	*Columba livia*	✓	✓	✓	✓	G	R
37	Rose-ringed parakeet	*Psittacula krameri*	✓	✓	✓	✓	F	R
38	Rufoustreepie	*Dendrocitta vagabunda*	-	✓	✓	✓	O	R
39	Scaly-breasted munia	*Lonchura punctulata*	-	✓	-	✓	G	R
40	Shikra	*Accipiter badius*	✓	-	✓	✓	C	R
41	Spotted dove	*Spilopelia chinensis*	✓	✓	✓	✓	G	R
42	Spotted owlet	*Athene brama*	-	-	✓	-	C	R
43	Tree pipit	*Anthus trivialis*	✓	✓	-	-	I	R
44	White-throated kingfisher	*Halcyon smyrnensis*	✓	✓	✓	-	C	R
45	Wire-tailed swallow	*Hirundo smithii*	✓	✓	✓	-	G	R
46	Yellow-legged green-pigeon	*Treron phoenicopterus*	-	-	-	✓	F	R

F=Frugivores; O=Omnivorous; N=Nectarivores; G= Granivores; C=Carnivores; I= Insectivorous; LC=Least Concern; R=Resident; SM=Summer Migrant; WM=Winter Migrant

Table 2. Association of bird species with tree species at selected locations

Tree Species → / Bird Species ↓	Banyan (*Ficus benghalensis*)	Chinaberry (*Melia azedarach*)	Cluster Fig (*Ficus racemosa*)	Black Plum (*Syzygium cumini*)	Mango (*Mangifera indica*)	Peepal (*Ficus religiosa*)	Pilkhan (*Ficus virens*)
	Indian robin	House crow (L1, L3, L4)	Brown-headed barbet	Red-vented bulbul (L1)	House crow(L1)	Common myna (L1)	Asian koel
	Red-vented bulbul	Common myna (L1, L3)	Brahminy starling	Common tailorbird (L1, L4)	Purple sunbird (L1)	Red-vented bulbul (L2)	Rufoustreepie
	Common hoopoe	Red-vented bulbul (L1, L4)	Rose-ringed parakeet		Red-vented bulbul (L1, L2, L3)	House crow (L2)	Indian silverbill
	Black drongo	Rock pigeon (L2, L3)	Purple sunbird		Common tailorbird (L1, L3)	Common hoopoe (L4)	
	Black kite	Eurasian collard dove (L3)			House sparrow (L2, L4)	Yellow-legged green pigeon (L4)	
	Asian koel	Jungle babbler (L4)			Jungle babbler (L2)		
	Purple sunbird	Oriental magpie robin (L4)			Oriental magpie robin (L2)		
	Lesser golden-backed woodpecker						
	Indian grey hornbill						
	Common hawk cuckoo						

Table 3. Correlation coefficient: feeding guilds vs structural features

Structural features	Carnivores	Frugivores	Granivores	Insectivores	Nectarivores	Omnivores
Road	0.93	-0.88	0.10	0.95	0.22	-0.33
Vegetation	-0.48	0.91	-0.68	-0.87	-0.49	0.66
Buildup	0.05	-0.64	0.79	0.56	0.46	-0.62
Fellow land	-0.22	0.59	-0.15	-0.73	0.16	0.04
Park	0.81	-0.96	0.34	1.00	0.33	-0.47
Species Richness	-0.31	0.55	-0.92	-0.29	-1.00	0.98

distribution, composition and structure were affected by urbanization in Ludhiana city (Van Heezik et al., 2008). Kler (2006) recorded 29 bird species belonging to 15 orders from locations selected on periphery of the city. The present study showed that increase in urbanization affected the bird species richness, in which urban habitats were dominated by urban generalist species; thus, creating biotic homogenization as corroborated by Pal et al. (2019). Marzluff (2005) stated that extreme disturbances caused synanthropic bird species to become dominant. Heterogeneity in avifaunal composition was accentuated by Bray-Curtis dissimilarity index (Bray and Curtis, 1957) showing maximum difference between location 1 and 3. Presence of woodpeckers was only recorded at location 4 dominated by *Ficus* tree species providing sites for nesting. Urban areas having multistory buildings, high windows provide an easy site for nesting and also safeguarding the nests from predators (Akram et al., 2015). Frugivores were observed to be most abundant at location 4 with maximum vegetation. In the present study, urbanized area was numerically dominated by granivoresas well as omnivores as multistory buildings and window cliffs offered nesting sites and artificial food provisioning as corroborated by Pal et al. (2019) mentioning that species like common myna and red-

vented bulbul also showed higher abundance values in studied urban areas which are likely to get benefitted from urbanization. Tresch et al. (2019) concluded that management actions in urban areas should improve landscape heterogeneity or reduce buildup area. Urban locations having balanced buildup area and vegetation might help in sustaining higher bird species richness and diversity in feeding guilds as found in studied location 3. Stessens et al. (2020) laid stress on appreciation of remote sensing techniques for urban design, planning and policy intervention which were also been corroborated by present study findings. The present study concluded that avian diversity and diverse feeding guilds were positively correlated to area under vegetation as compared to other habitat components utilizing geospatial technologies and field observations. Therefore, remote sensing and geospatial technologies might play a significant role in identifying urban areas for avian conservation.

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DNA BARCODING OF MAJOR INSECT PESTS AND THEIR NATURAL ENEMIES FROM CUCURBITACEOUS CROPS IN NORTHEAST INDIA

Arensungla Pongen*, G T Behere[1], D M Firake[1], Bhagawati Sharma[1] and T Rajesh

School of Crop Protection, College of Post Graduate Studies, (Central Agricultural University), Umiam 793103, Meghalaya, India
[1]Division of Crop Protection, ICAR Research Complex for NEH Region, Umiam 793103, Meghalaya, India
*Email: arensungpongen@gmail.com (corresponding author)

ABSTRACT

Correct identification of insect pest is a prerequisite for any control measures, and DNA barcoding facilitates this. In this study, assigning of 28 specimens (insect pests and natural enemies) to known species using DNA barcode by sequencing partial cytochrome oxidase I (COI) gene of mitochondrial DNA has been accomplished. Quick identification of a non-indigenous species *Bactrocera cilifera* (Diptera: Tephritidae) in Meghalaya has been enabled and taxonomic ambiguity of *Henosepillachna pusillanima* (Coleoptera: Coccinellidae) resolved. Molecular identity of *Malcus* sp., *Paridea* sp. and *Coridius* sp. has been established with NCBI GenBank registrations.

Key words: Cucurbits, insect pests, natural enemies, species identification, COI gene, sequencing, non-indigenous, ambiguity, DNA barcodes, molecular identity.

Cucurbits are widely cultivated in India, and northeast India is known for its good quality of produce. Insect pests' infestation and yield loss from 30 to 100% to cucurbits is known from different parts of the world (Dhillon et al., 2005). Besides insect pests, several natural enemies also harbour cucurbit ecosystems. Some of these provide biological control against insect pests and keeping them below economic injury level (Chambers and Adams, 1986) and help the farmers (Gul et al., 2017). With millions of insect species and their various lifestages, correct identification becomes a challenge for taxonomy (Zhang, 2011). The accurate taxonomic identification is an essential step before implementing any control measures. On the other hand, misidentifications could lead to ineffective management (Rivera and Currie, 2009), and there is a dire need to accelerate species discovery with new initiatives which the advancement of technology has to offer (Godfray, 2002; Hebert et al., 2003; La Salle et al., 2009).

The north eastern (NE) region of India comprises of eight states namely Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim, is one of the biodiversity hot spots of India. Its uniqueness lies in its sharing international borders with China, Bhutan, Myanmar, Bangladesh and Nepal (Gogoi et al., 2009), making transboundary insect migration inevitable (Behere et al., 2007). Most of these borders are porous and the quarantine setup is almost poorly maintained. Due to the remoteness, the resources are not properly explored and as a result, little information is available on insect diversity (pests and natural enemies), especially in cucurbits ecosystem. With the advances in science, it is now possible to facilitate the identification of new or invasive species very quickly using various molecular techniques (Behere et al., 2008). Amongst the molecular techniques, DNA barcoding is gaining attention for identification of taxonomically difficult species concisely. It is a taxonomic method that uses mitochondrial COI gene which is a short genetic marker in an organism's DNA in order to identify a particular species (Hebert et al., 2003). Comprehensive molecular information on insect pests and natural enemies of cucurbit crops is very limited as India has generated a total of only 4.6% barcodes of known species with its contrast to an approximate of 59,000 described insect species. On the other hand the corresponding global scenario is about 16% of described species, therefore a lot of emphasis is required to catch up with the world scenario (Jalali et al., 2015). Considering these, the present study analyses the insect pests and their natural enemies in cucurbits through species specific DNA barcodes using mtCOI gene.

MATERIALS AND METHODS

This study was carried out during 2017-2018 in the insect molecular biology laboratory of ICAR (Indian

Council of Agricultural Research) research complex for Northeastern Hills (NEH) Region, Meghalaya. Experimental farms of ICAR research complex and College of Post Graduate Studies (CPGS), Umiam, Meghalaya (25°41'N,91°55'E) supported the field work. Insects were collected from the major cucurbitaceous crops viz., pumpkin (*Cucurbita maxima*), cucumber (*Cucumis sativus*), bottle gourd (*Lagenaria siceraria*), spine gourd (*Momordica dioica*) and chow-chow (*Sechium edule*). The samples were collected by various methods (hand picking, net sweeping, aspirator) and stored in clean glass vials. The parasitoids were either collected directly or with rearing parasitized insect pests The collected specimens were either dry preserved in boxes or wet preserved in 70% ethanol in vials after labelling, the latter were preserved at -20°C. Voucher specimens have been deposited at the Insect Museum of Entomology Section of Crop Protection Division, ICAR Research Complex for North Eastern Hill (NEH) Region, Umiam, Meghalaya.

Genomic DNA (gDNA) was extracted from two specimens of each species (a single leg or antennae in case of large insect and whole insect in case of small insects) using modified phenol: chloroform protocol (Behere et al., 2007). These were tested for presence of *Wolbachia* infection using *Wolbachia* genes specific primer viz., Wol16SF/Wol16SR (O'Neill et al., 1992) and WSP81F/WSP96R (Zhou et al., 1988); PCR protocol was followed according to the composition and profile described by Murthy et al. (2011). The detection of *Wolbachia* was done prior to DNA barcoding as the presence of *Wolbachia* DNA in total genomic extracts made from insects is unlikely to compromise the accuracy of the DNA barcode library (Jalali et al., 2015). For mtCOI gene-based barcoding, PCR amplification was carried out in the thermal cycler (Eppendorf, India) to test the amplification of all the sample using a partial 709 bp cytochrome oxidase I (COI) gene base marker LCO/HCO (Folmer et al., 1994) and LepF1/LepR1 (Hebert et al., 2004). The reaction mixture contained 2µl of gDNA (~40-50 ng), 0.5µl each of forward and reverse primers, 5µl of ready to use EmeraldAmp® MAX PCR Master Mix (2x) (Takara) and 2µl of molecular biology grade water. The standard PCR profile consisted of one cycle of 2 min at 94°C, 5 cycles of 30 s at 94°C, 40 s annealing at 45°C, 1 min extension at 72°C, followed by 35 cycles of 94°C for 30 s, 51°C for 40 s and 72°C for 1 min. A final extension was allowed for 10 min at 72°C and samples were allowed to hold at 10°C in PCR machine after completion of all the cycles and then stored in -20°C for further use. Gel electrophoresis was performed using 1.5 % agarose to detect the genomic DNA using gel documentation (Care stream Gel Logic 212 Pro). The amplified products were got sequenced by M/s Eurofins Genomics India Pvt. Ltd, Bangalore, India. Sequencing was performed for all the samples from both the ends (5' and 3'). The DNA sequences were analyzed using the Molecular Biology software, Staden Package (Staden, 2000) under pregap and gap mode. Thereafter, Basic Local Alignment Search Tool (BLAST) search in online portal of National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) was conducted for identity and homology of all the analyzed sequences. The representative sequence of partial COI gene of species identified was deposited with NCBI and accession numbers obtained. All sequences were uploaded to GenBank and Barcode of Life Data (http://www.boldsystems.org). The DNA barcode images of the sequences submitted were developed using web based software http://www.cib.res.in/ibin/create-barcode.pzhpavailable at Insect Barcode Informatica (IBIn), ICAR-NBAIR, Bengaluru, India.

RESULTS AND DISCUSSION

A total of 31 insect species were observed in the study, classified under six orders viz., Coleoptera (12), Hemiptera (7), Diptera (4), Lepidoptera (3), Hymenoptera (4) and Araneae (1) (Table 1). These results corroborate with those on arthropods associated with cucurbits reported from other regions (Gameel, 2013; Vinutha et al*.,* 2017). The collected insect pests were preliminary identified based on known taxonomic keys and in cases of ambiguities, the insect specimens were sent to ICAR-Indian Agricultural Research Institute (IARI), New Delhi; University of Agricultural Sciences (UAS), Bengaluru; ICAR- National Research Centre for Banana, Tiruchirappalli, Tamil Nadu. The analyses of bacterial endosymbiont *Wolbachia* confirmed the fact that in the reproductive tissues of arthropods, as many as 25 to 70% of all insect species are potential hosts (Werren and Windsor, 2000). Three species viz., *Diadegma* sp., *Diachasmimorpha* sp. and *Hyposoter* sp. resulted positive and thus were not further used (Table 1). Multiple specimens were subjected to this step and those specimens which resulted positive were discarded.

The DNA barcode was successfully developed for 28 species by sequencing partial mtCO1, and sequencing analysis was carried out utilizing the pregap and gap program within the software staden

Table 1. Details of species along with barcoding and NCBI accession numbers

Name of insect species	Order: Family	Insect status	Host	Wol16SF Wol16SR	WSP81F WSP96R	nt. length (bp) : Protein length	Accession number
Bactrocera cilifera	Diptera: Tephritidae	Pest	Spine gourd	-ve	-ve	663: 215	MH395849
Leptoglossus gonagra	Hemiptera: Coreidae	Pest	Spine gourd	-ve	-ve	669: 216	MH395857
Aulacophora lewisii	Coleoptera: Chrysomilidae	Pest	Gourds, pumkin, cucumber	-ve	-ve	673: 224	MH198035
Aulacophora foveicollis	Coleoptera: Chrysomilidae	Pest	Gourds, pumpkin, cucumber	-ve	-ve	536: 178	MH198036
Paridea sp.	Coleoptera: Chrysomilidae	Pest	Gourds, pumpkin, cucumber	-ve	-ve	639: 213	MH198026
Tiracola plagiata	Lepidoptera: Noctuidae	Pest	Chow chow	-ve	-ve	647: 210	MH395862
H. pusillanima (12 spots)	Coleoptera: Coccinellidae	Pest	Gourds, pumpkin, cucumber, chow-chow	-ve	-ve	650: 212	MH395853
H. pusillanima (14 spots)	Coleoptera: Coccinellidae	Pest	Gourds, pumpkin, cucumber, chow-chow	-ve	-ve	650: 216	MH395854
H. pusillanima (16 spots)	Coleoptera: Coccinellidae	Pest	Gourds, pumpkin, cucumber, chow-chow	-ve	-ve	596: 194	MH395855
Bactrocera cucurbitae	Diptera: Tephritidae	Pest	Bottle gourd, cucumber, pumpkin, chow-chow	-ve	-ve	666: 221	MH198034
Bactrocera tau	Diptera: Tephritidae	Pest	Bottle gourd, cucumber, pumpkin, chow-chow	-ve	-ve	591: 193	MH395850
Bactrocera carambolae	Diptera: Tephritidae	Pest	Bottle gourd, cucumber, chow-chow	-ve	-ve	640: 207	MH395848
Nezara viridula	Hemiptera: Pentatomidae	Pest	Cucumber, spine gourd	-ve	-ve	626: 208	MH198029
Mylabris sp.	Coleoptera: Meloidae	Pest	Pumpkin	-ve	-ve	637: 212	MH198030
Arthrotus flavocincta	Coleoptera: Chrysomellidae	Pest	Bottle gourd, pumpkin	-ve	-ve	642: 213	MH198037
Coridius sp.	Hemiptera: Pentatomidae	Pest	Cucumber, chow - chow	-ve	-ve	561: 180	MH395852
Oenopia sexareata	Coleoptera: Coccinellidae	Predator	Aphids	-ve	-ve	497: 165	MH198027
Apanteles sp	Hymenoptera: Braconidae	Parasitoid	Cucumber moth	-ve	-ve	670:218	MH395863
Oxyopes sp.	Araneae Oxyopidae	Predator	General predator	-ve	-ve	600: 197	MH395859
Bothrogonia tibetana	Hemiptera: Cicadellidae	Pest	Cucumber, pumpkin	-ve	-ve	669: 223	MH198033
Kolla paulula	Hemiptera: Cicadellidae	Pest	Cucumber	-ve	-ve	590: 194	MH395856
Spilarctia sp.	Lepidoptera: Eribidae	Pest	Pumpkin	-ve	-ve	667: 222	MH198025
Malcus sp.	Hemiptera: Malcidae	Pest	Cucumber	-ve	-ve	630: 204	MH395858
Micraspis sp.	Coleoptera: Coccinellidae	Predator	Aphids	-ve	-ve	589: 196	MH198031
Oenopia kirbyi	Coleoptera: Coccinellidae	Predator	Aphids	-ve	-ve	609: 203	MH198028
Coccinella septempunctata	Coleoptera: Coccinellidae	Predator	Aphids	-ve	-ve	523: 170	MH395851
Anadevidia peponis	Lepidoptera: Noctuidae	Pest	Bottle gourd	-ve	-ve	678: 220	MH395845
Aphis gossypii	Hemiptera: Aphididae	Pest	Cucumber, bottle gourd, pumpkin	-ve	-ve	669: 217	MH395846
Diachasmimorpha sp.	Hymenoptera: Braconidae	Parasitoid	Fruit flies	+ve	+ve	-	-
Diadegma sp.	Hymenoptera: Ichneumonidae	Parasitoid	Cucumber moth	+ve	+ve	-	-
Hyposoter sp.	Hymenoptera Ichneumonidae	Parasitoid	*Euproctis* sp.	+ve	+ve	-	-

package and the messy/ambiguous 5' and 3' end of the sequences were trimmed to obtain good quality sequence. The total length of the final sequence varied from species to species and it ranged between 497-678bp. The final analysed sequences were submitted to GenBank maintained by NCBI, with accession number (Table 1). DNA barcoding on insect pests of agricultural importance has led to identifying cryptic and potentially new species (Seifert et al., 2007; Vaglia et al., 2008; Burns et al., 2008). This fact is in line with the present finding of a non indigenous species *B. cilifera* in Meghalaya, which is a recently discovered fruit fly in India (Nair et al., 2017); also the taxonomical ambiguity in the identity of three species of the genus *Henosepilachna* with 6 spots, 7 spots and 8 spots on each elytron (Naz et al., 2008) was resolved. These results revealed that the barcoding detected no variation, and the sequences from these specimens were 100% identical to *H. pusillanima*. Over the last decade DNA barcoding has proven to be an authentic and efficient tool achieving species level resolution in 95 % to 97% of cases (Hebert et al., 2004; Ward et al., 2005). All the analysed sequences were subjected to BLAST and results with 99-100% homology to NCBI database were considered as similar species and molecular identity of the test species was confirmed.

However, for those species with blast result below 99%, the identity was established till genus level only (Table 1). The molecular identity of three species viz**.,** *Malcus* sp*., Paridea* sp. and *Coridius* sp. was established and the sequences have been deposited for the first time in the NCBI database. Hajibabaei et al. (2007), Carvalho et al. 2008 and Smith et al. (2008) corroborate with the potential of DNA barcoding results of the present study.

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AUTHOR CONTRIBUTION STATEMENT

GTB, DMF and AP conceived the research work plan. AP, GTB and BS conducted the experiment. DMF and TR contributed in identification. AP wrote the manuscript. All authors approved the manuscript.

CONFLICT OF INTEREST/ COMPETING INTERESTS

There is no conflict of interest/competing interests.

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EFFECT OF DIETARY AZASTEROIDS ON THE GROWTH AND DEVELOPMENT OF THE GRAM POD BORER *HELICOVERPA ARMIGERA* (HUBNER)

Rita Rath, Ranjana Saxena*, Versha Goel[1] and Renu Gupta[2]

Department of Zoology, Dyal Singh College;
[1]Maitreyi College; [2]Ramjas College; University of Delhi, Delhi 110007, NCT Delhi, India
*Email: ranjana.saxena@dsc.du.ac.in (corresponding author)

ABSTRACT

Effect of two azasteroids, 25-azacholestane and 25-azacoprostane was studied on growth and development of an economically important phytophagous pest, *Helicoverpa armigera* (Hubner) causing extensive damage to crops like cotton, pigeon pea, chickpea and others. 25-Azacholestane caused an increase in larval mortality (36% in control to 52% at 25 ppm), decrease in pupation (64% in control to 48% at 25 ppm) and decrease in adult emergence (60% in control to 28% at 25 ppm). Larval and pupal duration was significantly more as the azacholestane concentration increased to 25 ppm ($p<0.001$). Similar results were observed in case of 25-azacoprostane treatment also. Both the azasteroids were found to have inhibitory effect on the growth and development of *H. armigera*. Formation of larval-pupal intermediates and adults with abnormal wings was also observed. Addition of 0.1% cholesterol along with 10 ppm of 25-azacoprostane in the diet reversed the inhibitory effect of the azasteroid.

Key words: *Helicoverpa armigera*, 25-azacholestane, 25-azacoprostane, sterol, cholesterol, larval duration, larval mortality, pupal duration, pupal mortality, adult emergence, metabolism, utilization, inhibition, IPM.

One of the basic tenets of insect biochemistry is the inability of insects to synthesize cholesterol de novo (Jing and Behmer, 2020; Li and Jing, 2020). Insects fulfil their cholesterol requirements from dietary sources which in case of phytophagous insects is the plant sterols (Rath and Agarwal, 1988; Jing and Behmer, 2020). Dominant plant sterols such as sitosterol, stigmasterol and campesterol are converted to cholesterol by the dealkylation pathway using several enzymes (Svoboda and Feldlaufer, 1991; Svoboda et al., 1995; Behmer et al., 1999). Cholesterol is essential for growth and development of insects being a constituent of cell membranes and as a precursor for ecdysteroid synthesis (Svoboda and Thompson, 1985; Tarlochan et al., 1998; Gilbert et al., 2002; Behmer and Nes, 2003; Toprak et al., 2020; Entringer et al., 2021; Goel et al., 2021). Azasteroids are known to inhibit the pathway of conversion of phytosterols to cholesterol, thereby affecting growth, moulting and development in some insects (Svoboda and Robbins, 1971; Svoboda et al., 1972; Thompson et al., 1975; Agarwal et al., 1990). These compounds also inhibit phytosterol biosynthesis (Darnet, 2020), have antifungal properties (Burbiel and Bracher, 2013). Therefore, the role of these azasteroids on sterol metabolism pathways, targeting insect growth, could be many folds. In insects, the hormonal regulation of vital processes such as metabolism, moulting, reproduction and diapause are unique which is not found in other higher groups of animals. Compounds which could target these processes, have specificity and high biological activity to higher animals could be a promising candidate for safe insect pest management practices. However, limited literature is available regarding effects of azasteroids on *Helicoverpa armigera* (Hubner). Therefore, two azasteroids namely 25-azacholestane and 25-azacoprostane were studied for their effect on the growth and development in *H. armigera* , a major and economically important pest of cotton, pulses and many other crops worldwide (Ravi et al., 2005; Haile et al., 2021). These compounds were given in the artificial diet to study their effect on the larval duration, larval mortality, percent pupation, pupal duration, adult emergence and any other abnormality. The azasteroids or similar compounds, targeting the requirement of sterols in insects, could be exploited as an alternative method for pest management (Kuthiala et al.,1987; Agarwal et al., 1990; Svoboda and Weirich, 1995) and also for better understanding of sterol biology in other animals including humans (Jing and Behmer, 2020).

MATERIALS AND METHODS

The larvae of *H. armigera* used in these studies (procured from culture maintained in Department

of Zoology, University of Delhi) were reared on an artificial diet (Rath, 1988). Due to the cannibalistic nature of the larvae, they were reared individually in glass vials (7.5 cm h x 2.5 cm dia.) plugged with cotton containing 7-9 ml diet, till pupation (Rath and Agarwal, 1988). The culture of *H. armigera* was maintained in the laboratory under the controlled conditions of temperature, 26.1± 2°C, 70± 5% RH and a photoperiod regime of 16L:8D (Rath, 1988). On pupation, the pupae were sexed and kept in glass jars (20 cm h x15 cm dia.) till adult emergence and egg laying (5 to 10 pairs/jar). Cotton soaked with 10% honey solution was placed in glass vials and hung on the sides of the jar. The freshly hatched first instar larvae from the stock culture were then transferred and reared till adult emergence on an artificial diet same as the stock diet which was fortified with varying concentrations of the azasteroids. The rearing conditions remained same as the stock culture.

The azasteroids tested individually were 25-azacholestane and 25-azacoprostane (gifted by Dr J A Svoboda, Insect and Nematode Hormone Laboratory, USDA, Beltsville, Maryland, USA) ranging from 5 to 50 ppm of the wet weight of the diet in case 25-azacholestane and 5 to 25 ppm in case of 25-azacoprostane. Insects reared on stock diet without the azasteroids served as control. Minimum of 25 larvae were tested for each concentration and 7 to 9 ml of the test diet was given to each larva. Daily observations were made on the larval mortality, larval duration, pupal duration, % pupation and adult emergence. Any other morphological abnormalities seen were also recorded. Another set of experiment was conducted in which the freshly hatched larvae of *H. armigera* were reared on an artificial diet same as the stock but fortified with 10 ppm of 25-azacoprostane and 0.1% cholesterol (CDH, purity>99%) of the fresh weight of the diet. Daily observations were made of the various parameters as above for the growth and development of these insects. Statistical analysis of the data was done using Mann-Whitney Rank Sum test.

RESULTS AND DISCUSSION

When 25-azacholestane was added to the artificial diet in concentrations ranging from 0 to 50 ppm, it was seen that the larval mortality increased from 36% in the control to 52% at 25 ppm of 25-azacholestane (Fig. 1). However, a further increase in 25-azacholestane to 50 ppm caused a decrease in larval mortality. Pupation decreased from 64% in control to 48% at 25 ppm. At 50 ppm azacholestane the pupation again increased to 64% (Fig. 1). The adult emergence also showed a similar pattern of decrease till 25 ppm but at 50 ppm increased to 52%, though still less than the control (Fig. 1). The larval duration significantly increased in the groups whose diet was treated with 5, 10 and 25 ppm of 25-azacholestane ($p < 0.001$) as compared to the control group (Fig. 2). However, a further increase of azacholestane to 50 ppm in the diet did not show any significant difference with that of control. In both male and female, the pupal period increased significantly at 5 and 10 ppm azacholestane ($p < 0.001$) as compared to the control. In case of male pupae, though the duration decreased at 25 and 50 ppm azacholestane as compared to 5 and 10 ppm, it was still significantly higher than control ($p = 0.045$) at 25 ppm. While no significant difference was observed at 50 ppm ($p = 0.094$). In female pupae, similar pattern like male pupae was observed, however, the duration was significantly higher at both 25ppm ($p = 0.009$) and 50 ppm ($p < 0.001$) of azacholestane as compared to the control.

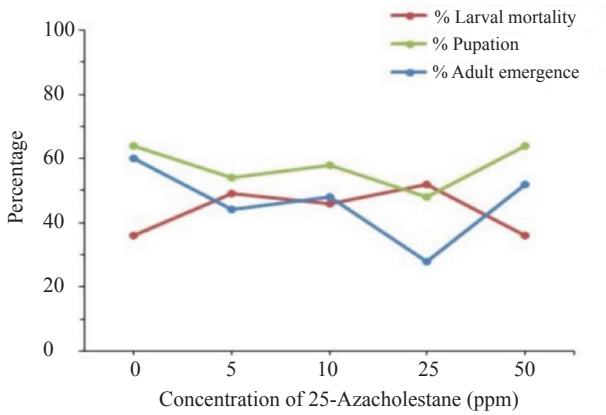

Fig. 1. Effect of 25-azacholestane on growth and development of *Helicoverpa armigera*

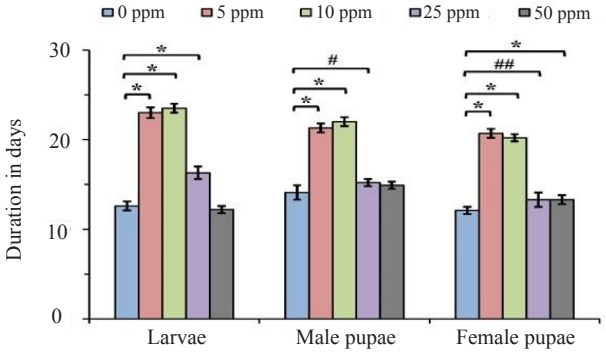

Fig. 2. Larval and pupal duration of *H. armigera* reared on an artificial diet containing 25-azacholestane. The data is presented in the bar graphs as mean± S.D. Statistical analysis was done by Mann-Whitney Rank Sum Test between the control and other groups for larvae, male pupae and female pupae individually *$p < 0.001$, ##$p < 0.01$, #$p < 0.05$.

Figures 3 and 4 show the effect of the second azasteroid, 25-azacoprostane on growth in *H. armigera*. When 25-azacoprostane was added to the diet at concentrations ranging from 0-25 ppm, the larval mortality increased from 10% to 27.3%. However, when 0.1% cholesterol was additionally provided in the diet containing 10 ppm of 25-azacoprostane, there was a reduction in the larval mortality to 16% (Fig. 3). The azacoprostane treatment caused a decrease in % pupation and adult emergence as compared to the control. In the presence of additional 0.1% cholesterol, the pupation and adult emergence increased to 84% and 72% respectively (Fig. 3). The larval duration significantly increased in the groups whose diet was treated with 5, 10 and 25 ppm of 25-azacoprostane ($p < 0.001$) as compared to the control group (Fig. 4).

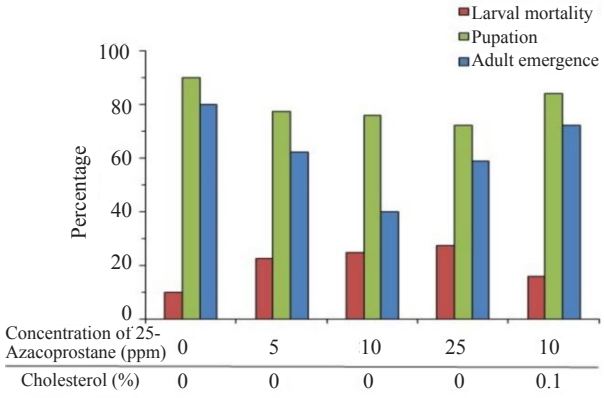

Fig. 3. Larval mortality, pupation and adult emergence of freshly hatched *H. armigera* larvae reared on an artificial diet containing 25-azacoprostane and cholesterol

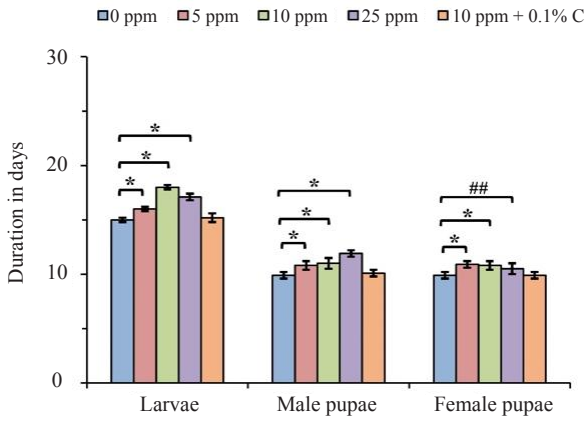

Fig. 4. Larval and pupal duration of *H. armigera* reared on artificial diet containing 25-azacoprostane and cholesterol**.** The data is presented in the bar graphs as mean± S.D. Statistical analysis was done by Mann-Whitney Rank Sum Test between the control and other groups for larvae, male pupae and female pupae individually.
*$p < 0.001$, ## $p < 0.01$.
C – Cholesterol

(a) dorsal view (b) lateral view

Fig. 5. Larval-pupal intermediate of *H. armigera* reared on an artificial diet containing 25-azacholestane or 25-azacoprostane

However, when 0.1% cholesterol was added along with 10 ppm of 25-azacoprostane in the diet, no significant difference in larval duration was observed as compared to control. The pupal period , in both male and female increased significantly with 5 ppm ($p < 0.001$), 10 ppm ($p < 0.001$) and 25 ppm (male pupae: $p < 0.001$; female pupae: $p = 0.009$) azacoprostane as compared to the control. In both male and female pupae, no significant difference in pupal duration was observed when 0.1% cholesterol was added with 10 ppm azacoprostane as compared to the respective control groups (Fig. 4). It was also observed that the presence of the azasteroids in the diet resulted in larval-pupal intermediates (Fig. 5) and abnormal wing formation in adults.

Compounds such as azasteroids are known to inhibit the metabolism of sterols in plants (Darnet et al., 2020), nematodes (Choi et al., 2003) and insects either by acting directly on the enzyme Δ^{24}-sterol reductase or at some other step in sterol metabolism, transport or utilization (Svoboda and Weirich, 1995). In insects, they block the formation of the moulting hormone or inhibit sterol transport and utilization which is essential for insect growth and development (Entringer et al., 2021; Toprak and Musselman, 2021). A retardation in growth and development due to azasteroids similar to that seen in our study on *H. armigera* has been reported in many insects (Al- Izzi and Hopkins, 1982; Kuthiala et al., 1987; Goel and Agarwal, 1987; Agarwal et al.,1990) and nematode, *Caenorhabditis elegans* (Choi et al., 2003). In some insects growth and development of the larvae was not inhibited even though the analyses of the sterols revealed that the Δ^{24}-sterol reductase enzyme was inhibited considerably. It appears then that a significant limitation in availability of cholesterol was not sufficient in itself to disrupt normal development but in addition ecdysteroid biosynthesis or metabolism might also be affected (Svoboda et al., 1972; Tarlochan et al., 1998; Gilbert et al., 2002; Entringer et al., 2021). Larval-pupal intermediates and abnormal wing formation in adults due to azasteroid treatment also indicate that the growth,

development and moulting of *H. armigera* is inhibited by these azasteroids. Similar results have been reported in other insects like *Diatraea grandiosella* (Chippendale and Reddy, 1973), *Anthonomous grandis* (Earl et al., 1967), *Epilachna varivestis*. (Walker and Svoboda, 1973), *Spodoptera litura* (Kuthiala *et al*, 1987). However at a higher concentration of 50 ppm of azacholestane, the larval mortality decreased in *H. armigera*. A lesser inhibitory effect of 25-azacholestane at higher doses is still obscure and may need further investigations. However, 25-azacoprostane had no effect on honey bee growth, development, sterol utilization or metabolism (Svoboda et al., 1987). When cholesterol was added to the diet in addition to the normal sterol content of the diet and 10 ppm of 25- azacoprostane, the growth of *H. armigera* was similar to that of control. This may indicate that the inhibitory effects of azasteroids on the growth of *H. armigera* could be a result of the reduction in the amount of cholesterol available to the insect due to azasteroids. This could be due to the inhibition of the enzyme Δ^{24}-sterol reductase and hence when additional cholesterol was added to the diet, the inhibitory effect of the azasteroid was nullified.

Similar studies of addition of cholesterol completely nullifing the inhibitive effects of 25-azacholesterol, has been reported in *Epilachna varivestis* (Walker and Svoboda, 1973). However, addition of cholesterol did not reverse the inhibitive effects of 25-azacholesterol on the boll weevil, *Anthonomus grandis* Boheman (Earl et al., 1967) and the housefly, *Musca domestica* (Svoboda et al., 1972). The ability of steroidal moulting hormone inhibitors or similar compounds to disrupt the pathway of sterol utilization and metabolism in agricultural and medicinal pests can be exploited for developing novel insect pest management practices (Yang et al., 2016; Entringer et al., 2021). Our studies also suggest azasteroids to have potential to inhibit sterol utilization and metabolism pathways as observed by the inhibitive effect on the growth and development of *Helicoverpa armigera*. The studies and investigations for alternative pest management strategies in general and for *H. armigera* in particular is an ongoing process. The use of biopesticides (Agale et al., 2021), botanical and semiochemicals (Edosa, 2019) and our present study using azasteroids could be explored as one of the futuristic approaches for pest management. Therefore, such studies with azasteroids and similar compounds along with field validations can open avenues to be exploited for safe pest management technologies. Research and studies on sterol metabolism and its inhibition in insects may also help to elucidate the knowledge of comparative biochemical and physiological processes of steroids in other organisms including humans.

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AUTHOR CONTRIBUTION

RR, RS, VG and RG helped to design and perform the experiments. RS and VG prepared the figures and analysed the results. RR wrote the manuscript. All authors have read the manuscript and agree to its publication.

CONFLICT OF INTEREST

RR, RS, VG, RG hereby declare that there is no conflict of interest.

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OCCURRENCE AND SPREAD OF INVASIVE THRIPS *THRIPS PARVISPINUS* (KARNY) IN NORTH INDIA

Timmanna H*, C Prashantha[1], P R Shashank, V D Nigam[1] and Narendra Birla[2]

Division of Entomology ICAR-Indian Agriculture Research Institute, New Delhi 110012, India
[1]Regional Central IPM Centre, Directorate of Plant Protection, Quarantine & Storage, Faridabad 121001, Haryana, India
[2]Regional Station, Indore, ICAR- Indian Agriculture Research Institute, New Delhi 110012, India
*Email: thimsento@gmail.com (corresponding author): ORCID ID 0000-0001-9399-4822

ABSTRACT

The invasive thrips *Thrips parvispinus* (Karny) (Thysanoptera: Thripidae) is an important insect pest causing severe damage in chilli-growing areas of south India in the last two years. In the present study, its spread in north and central India in chilli and capsicum is discussed along with diagnosis, occurrence, damage symptoms. A high level of infestation (ranging from 8-12 thrips/ flower) was observed on chilli crop in Madhya Pradesh (Khargone, Khandwa, Satna, Narasinghpur, Dhar) and on capsicum hybrids in Haryana (Ambala, Panchkula and Panipat) grown under greenhouse. However, its infestation was less (1-3 thrips/ flower) in chilli crop at Chhattisgarh (Bastar, Sarangarh-Bilaigarh, Balod bazar, Durg).

Key words: *Thrips parvispinus*, infestation, Madhya Pradesh, Haryana, Chhattisgarh, chilli, greenhouse, damage, diagnosis, quarantine pest

The invasive thrips, *Thrips parvispinus* (Karny) (Thysanoptera: Terebrantia: Thripidae) is native to Thailand and has widespread occurrence in other South East Asian countries (Mound and Collins, 2000). Hence, it is called South East Asian thrips. As compared to other thrips species, this is larger in size, with dark brown to black coloured body. It is primarily sap sucking insect, but also feeds on pollens and resides in flowers and curled leaves of chilli, capsicum, and other crops. The international trade of planting material and changing climate are considered major factors for its rapid spread to other countries like Oceania, North America, Europe, Africa, and now India (Sugano et al., 2013; EPPO, 2022; Rachana et al., 2021). Large-scale cultivation of its major host plants (solanaceous crops and papaya), and tropical weather conditions favour its further spread and establishment in new areas (Sartiami and Mound, 2013; Johari, 2015).

In India, *T. parvispinus* infestation was initially observed in papaya (*Carica papaya* L) in Bangalore during 2015 (Tyagi et al., 2015). Later it has been observed on other host plants viz., *Brugmansia* sp., *Tagetes* sp., *Citrullus lanatus*, *Momordica charantia*, *Chrysanthemum* sp., *Gossypium* sp., *Mangifera indica*, *Tamarindus indica*, *Dahlia rosea* and *Capsicum annum* (Rachana et al., 2021; Roselin et al., 2021). During 2021, post-rainy season, a higher level of infestation (10 to 20 thrips/ flower) was recorded in >10 lakh acres of chilli crop in Andhra Pradesh, Telangana, and Karnataka. Thus this pest is a threat to chilli growers of other states and in February 2022, showing 40 to 80% damage in most of the chilli growing areas of Andhra Pradesh and Telangana. (Anonymous, 2021; 2022; Timmanna et al., 2022). In view of these, monitoring this pest through regular surveys has been done in different states of north and central India. This study for the first time, reports its occurrence, along with its diagnosis in different chilli growing areas of north and central India.

MATERIALS AND METHODS

During the last week of November, chilli growing farmers from north Indian states like Haryana, Chhattisgarh and Madhya Pradesh observed severe incidence of large black thrips on the flowers and leaves of chilli. After this observation, separate roving surveys were conducted by a team of scientists from the Directorate of Plant Protection, Quarantine and Storage, Faridabad, Haryana (DPPQ&S) and ICAR-Indian Agricultural Research Institute (IARI), New Delhi. Thrips-infested capsicum crop (70-90 days old crop) grown under protected condition (nethouse) of Ambala, Panipat and Panchkula districts of Haryana were visited. Thrips infested chilli crop in Khargone, Khandwa, Satna, Narasinghpur and Dhar districts of Madhya Pradesh were also surveyed; also chilli plant samples

were received from farmer fields of Bastar, Sarangarh-Bilaigarh, and Balod Bazar districts of Chhattisgarh. In all these, infestation was observed, from which infested plants were randomly selected. Terminal shoots, leaves and flowers from these were tapped on to a white paper sheet and fallen thrips were collected in vials containing 70% alcohol with a camel hair brush. For morphological identification slide mounts were prepared following the standard procedures and diagnosis was carried out under DM2500 LED compound microscope, using standard keys (Hoddle et al., 2012). The voucher specimens have been submitted to the National Pusa Collection, Division of Entomology, ICAR-IARI, New Delhi, India.

RESULTS AND DISCUSSION

Thrips samples from Madhya Pradesh, Chhattisgarh and Haryana states were confirmed as *Thrips parvispinus* Karny (Fig. 1. g, h). The important diagnostic characters observed include- female dark brown, head and thorax paler than abdomen, legs yellow; male pale brown to yellowish and smaller compared to females; with two pairs of long postero-angular, and three pairs of posterior marginal setae on the pronotum; campaniform sensilla on metanotum absent and reticulate medially; long median setae located behind anterior margin; male thrips yellowish with posteromarginal comb absent on VIII tergite; sternal segments III to VII having small transverse pore plate and discal setae at lateral. *Thrips parvispinus* has originated from Thailand and for the first time it was reported on papaya (*Carica papaya*) from Bangalore (India). Later, within a span of five years, it became serious in most of the chilli-growing areas of south India viz., Andhra Pradesh, Telangana, and Karnataka. In the present study, more immature and adults (ranging from 3 to 12 thrips/ flower) were noticed on flowers and leaf midribs of chilli (70 to 90 days old), which is grown in open field conditions, as seen from the five districts of Madhya Pradesh and four districts of Chhattisgarh (Table 1). Infested plants showed a silvery appearance to brownish discolorations on tender leaves and fruits, crinkling and upward curling of leaves, buds and leaves become brittle at a later stage, with more flower droppings (Fig. 1c-f). These symptoms are in conformity with those of previous ones known from south India (Anonymous, 2021; Rachana et al., 2022; Timmanna et al., 2022).

Table 1. Chilli black thrips *T. parvispinus* spread in central and north India

State	Location	Infestation (thrips/ flower)	Crop/ variety	GPS Coordinates
Madhya Pradesh	Khargone	8-12	Chilli (Navtej MHCP-319, Shakti 51)	21.866”N, 75.614”E
	Khandwa	8-10	Chilli (Shakti 51, Navtej MHCP-319, AK-47)	21.832”N, 76.351”E
	Dhar	7-8	Chilli (NAVTEJ MHCP-319, AK-47)	22.598”N, 75.306”E
	Satna	8-10	Chilli (Navtej MHCP-319)	24.608”N, 80.803”E
	Narsinghpur	6-8	Chilli (Navtej MHCP-319, AK-47)	22.948”N, 79.193”E
Chhattisgarh	Bastar	4-5	Chilli	19.208”N, 81.934”E
	Baloda bazar	4-5	Chilli	21.658”N, 82.158”E
	Sarangarh-Bilaigarh	3-5	Chilli	21.621”N, 83.113”E
	Durg	3-5	Chilli	21.401”N, 81.079”E
Haryana	Sambhalkha (Ambala)	8-16	Inspiration RZ F1 Red Capsicum Bachata RZ F1 Yellow Capsicum	30.294”N, 76.906”E
	Panipat	2-3	Capsicum	29.424”N, 77.015”E
	(Magniwala) Panchkula	2-3	Capsicum	30.746”N, 76.968”E

Fig 1. a. infested capsicum crop in nethouse (Sambhalkha village, Ambala district); b) infested plant; c, d. *T. parvispinus* load on flowers; e, f. infested crop (open field, Khandwa district); g. female adult; h. Male adult

The expert team from DPPQ&S observed these on the greenhouse grown capsicum crop at Sambhalkha village of Ambala district (up to16 thrips/ flower) in flowers and terminal leaves; however, comparatively less infestation (2 to 3 thrips/ flower) was observed in Panipat and Panchkula districts (Table 1; Fig 1). Severely infested plants showed brownish and yellowish discoloration on flowers and leaves, respectively; with stunted growth and deformed fruits. These observations corroborate with those observed in Southeast Asian countries (Sartiami and Mound, 2013; Johari, 2015). Since this pest is new to the Indian subcontinent, it is very difficult to predict its spread within India, probable reasons for its rapid spread might be the unrestricted movement of agricultural commodities and planting materials from infested nurseries (Shashank et al., 2016); availability of host plants around the year might be other reason (large scale cultivation of solanaceous and papaya crops); and subtropical weather conditions favour this (Sartiami and Mound, 2013; Johari, 2015; Baradevanal et al., 2021; Timmanna et al., 2022). The innate ability of pests viz., higher reproduction potential and aggressive feeding behaviour of *T. parvispinus*, might favour its multiplication, establishment, and further spread. Early detection with regular monitoring is the key for formulating sustainable management strategies for such invasive pests. The adoption of adhoc recommendation by DPPQ&S might help reduce its spread.

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AUTHOR CONTRIBUTION STATEMENT

TMH and CPR conceived and designed research. TMH, CPR, SPR, VDN and NBR conducted experiments. TMH & SPR wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

Authors declares, there is no conflict of interest.

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OBSERVATIONS ON THE PREDATION BY THE CRAB SPIDER *THOMISUS ONUSTUS*

S Shweta, Anil Kurmi[1] and Sandeep Kaushik[2]*

Department of Botany, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur, Chattisgarh 495001, India
[1]Krishi Vigyan Kendra, Indira Gandhi National Tribal University,
Amarkantak, Anuppur 484887, Madhya Pradesh, India
[2]Department of Environmental Sciences, Indira Gandhi National Tribal University,
Amarkantak, Anuppur 484887, Madhya Pradesh, India
*Email: sskaushik2002@igntu.ac.in (corresponding author): ORCID ID 0000-0002-2730-1618

ABSTRACT

Crab spider of the genus *Thomisus* Walckenaer (Araneae: Thomisidae), the *T. onustus* is a potent predator able to hunt prey larger than its own size. This study reports on a predatory event involving this spider, mimicking the *Cajanus cajan* flower for effective predation of pollinator bee *Apis dorsata*. The study focuses on the predation mechanism utilized by the spider making use of the plant architecture. The observations reveal that in a papilionaceous yellow coloured corolla of *C. cajan*, the same-coloured *T. onustus* is able to colour camouflage and can accommodate itself in a concealed position beneath the standard petal thus predating the prey by deceiving.

Key words: *Cajanus cajan*, cavae, keel petal, *Thomisus onustus*, crab spider, predation, *Apis dorsata*, camouflage, mimicry, prey, deceiving, corolla

Biotic interactions in the natural ecosystem range from symbiosis to parasitic associations where the organism has adapted itself for millions of years driving the energy chain of the ecosystem. Adaptations for predation have been successfully used by predators for capturing their prey in natural ecosystems. One of the most effective predations where the predator is unnoticed by the prey, thereby assuring that the prey would get trapped is mimicry and camouflage. During the course of time and various mechanisms for predation, a type of biotic interaction gets evolved for capturing the prey. Such an interaction is where the colouring seems to be a compromise between being conspicuous to conspecific or in other terms being poorly visible to predators or prey. Camouflage or mimicry is not only effective for escaping from their predators but also for deceiving the prey as they go unnoticed by the latter thereby easily trapping them. Thus, the predators ensure finding a prey while using flower as a platform for their feeding activities. Spiders are diverse groups of invertebrates and as per the World Spider Catalog > 47000 species and over 4000 genera are known. The crab spiders belonging to the genus *Thomisus* are known to have a mutualistic association with the plants. The spider resides in the flower and prevents or deters the harmful florivores or herbivorous insects. In turn, the plants are known to attract these spiders by emitting floral volatiles such as β-ocimene upon attack by herbivores (Knauer et al., 2018). Interestingly the spider uses the floral physical colour and structure as platform for carrying out its predatory activities. The crab spider also feeds on the pollen and nectar of the flower during the lean phase of pollinators and thus can survive for an extensive period (Vogelei and Greissl, 1989). In addition to it, when the female crab spiders mimic different flower species, they are simultaneously cryptic in the colour-vision systems of both bird predators and hymenopteran preys (Thery and Casas, 2002). The present paper is a report of crab spider *T. onustus* utilizing the flowers of *Cajanus cajan* for its predation.

MATERIALS AND METHODS

Field observations were carried out in *C. cajan* agricultural plots at the Indira Gandhi National Tribal University Amarkantak, Madhya Pradesh during the February 2022 (flowering stage of *C. cajan*) during the day when the pollinators visited the flowers. The predatory behaviour of the crab spider *T. onustus* was observed and the same was documented with photographs.

RESULTS AND DISCUSSION

A typical papilionaceous flower has a vexillum (standard) petal which encloses the alae (wings) and the carina (keel) petals (Fig. 1A). Since the colour of the flower and that of the crab spider *T. onustus* is

yellow, latter was able to disguise itself with the flower colour (Fig. 1B-D). Thus, the prey is unable to notice the spider due to cryptic colouration. Similar pattern has been observed in case of the Australian crab spider *Thomisus spectabilis* (Heiling et al., 2005). Moreover, the architectural pattern of the papilionaceous corolla also has a distinct advantage for the crab spider, which perhaps enhances its efficiency of predation as the spider is able to fit into the inner cavity of the standard petal (Fig. 1B-D), thus concealing itself from the visiting pollinator and even protects itself from its predators. Thus, in a papilionaceous corolla, the spider has a dual advantage, one being the colour camouflage and the second being the spiders gets into a concealed position beneath the standard petal. It seems that the spider is able to use the cavae present on the wing petals of *C. cajan* to get a firm grip (Fig. 1D). For creating enough space for the visiting pollinator, such as the honey bee, *Apis dorsata,* the spider pushes the standard petal to create an orifice (Fig. 1B-C). The honey bee visits the flower little realizing the consequences of entering the flower. It has been observed that the spider adopts sit-and-wait approach for its prey so that the spider will get its food since the pollinators are sure to visit the flowers for collection of the nectar as a reward for carrying out pollination. Even a single pollinator visiting the flower is enough for the spider to trap its prey. As soon as the bee forces its entry into the flower through the orifice, the spider ambushes it by using the raptorial forelegs preventing its escape, and ultimately leading to death (Fig. 1D). Subsequently, the spider feeds on the bee. A dipteran is also recorded on the prey *Apis* sp. (Fig 1-D). This is the first report of the crab spider *T. onustus* using *Cajanus cajan* flower as an effective site for predation.

There are reports of *T. onustus* predating many insects using flowers of many plants such as *Erigeron annuus* (L.) Pers., *Bellis perennis* L., *Glebionis segetum* (L.) Fourr., *Malva sylvestris* L., *Chrysanthemum frutescens* (L.) Sch.Bip., *Eryngium* L. sp., *Erica tetralix* L., *Biscutella laevigata* L., etc. (Llandres et al., 2012; Knauer et al., 2018; Rodríguez-Gironés and Jiménez,

Fig. 1. *Thomisus onustus* mimicking the flower of *C. cajan* for its predation; A. A typical papilionaceous corolla; B. *T. onustus* mimicking the corolla; C. *T. onustus* positioning itself under the vexillum of *C. cajan*. D. *T. onustus* ambushing the bee *Apis* sp. using the raptorial forelegs

2019;). Similar patterns of crab spiders belonging to the genus *Phrynarachne* deceiving its prey have been recorded (Yu et al., 2021). On the other hand, in case of *Epicadus heterogaster* it has been observed that the spider attracts pollinators regardless of flower thus representing an evolutionary pathway (Vieira et al,, 2017). However, in flowers where the papilionaceous corolla is absent, the chances of spider getting spotted increases, hence the pollinator becomes cautious and avoids visiting such flowers (Antiqueira and Romero, 2016); and due to which a reduction in the bee visits has been observed (Knauer et al., 2018). Papilionaceous flowers such as the one in *C. cajan* serves dual advantage for the crab spider for predating the pollinators. Not only the colour of the spider is the same with the flower colour but the crab spider also conceals itself in the inner cavity of the standard petal thereby getting conspecific from its predators as well as preys. The spider is able to utilize the cavae present on the keel petal to get a firm grip on the flower. The spider pushes the standard petal to create an orifice for the visiting bee. Thus, the present observations indicate that crab-spiders' colour mimicry works successfully on the visual systems of both predator and prey.

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COMPARATIVE BIOLOGY OF RICE SWARMING CATERPILLAR *SPODOPTERA MAURITIA* BOISDUVAL

Mogili Ramaiah* and T U Maheswari[1]

ICAR-Krishi Vigyan Kendra, Acharya N G Ranga Agricultural University,
Kalyandurg 515761, Andhra Pradesh, India
[1]College of Agriculture, PJTSAU, Rajendranagar, Hyderabad 500030, Telangana, India
*Email: ramaiahmogili@gmail.com (corresponding author): ORCID ID 0000-0002-2230-6567

ABSTRACT

Comparative biology of rice swarming caterpillar *Spodoptera mauritia* Boisduval has been analysed in this study. It was observed that rearing it on the artificial diets (chickpea flour based diets) was the most favourable with shortest larval period of 20.00± 0.51 days, whereas, those fed with rice leaves were 23.00± 0.52 days. The number of eggs laid was 1887.50 and 1013.50 when reared on artificial diet and rice leaves, respectively.

Key words: *Oryza sativa*, swarming caterpillar, *Spodoptera mauritia*, Noctuidae, biology, artificial diet, natural diet, mass rearing, larval period, fecundity

The rice swarming caterpillar, *Spodoptera mauritia* (Biosduval) (Lepidoptera: Noctuidae) is a serious pest of rice in Telangana, Odisha and Assam in the nursery stages (Pradhan and Jotwani, 1992; Sain and Prakash, 2008; Tanwar et al., 2010; Ramaiah et al., 2018; Banu et al., 2022). This pest has a tendency to migrate in large swarms, and grazes a field like cattle and hence it is referred to as army worm (Pradhan and Jotwani, 1992). The caterpillar is essentially a polyphagous and during the last few years, it has emerged as a major pest in eastern India (Tanwar et al., 2010). In Odisha, this pest has occurred over thousands of hectares in 2009, especially in Sambalpur and Sundergarh districts (Tanwar et al., 2010). The continuous maintenance of laboratory colony of insect species is needed for any biochemical and physiological studies. With the development of artificial diets, the mass rearing of insects has been facilitated. Large scale rearing of larvae is difficult in natural medium because there is need to change the diet frequently. Artificial diet for *S. mauritia* has not been standardized, and in the present study rearing of *S. maurita* on chickpea based artificial diet has been attempted and the comparative biology analysed.

MATERIALS AND METHODS

The study was done at the Department of Entomology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad during August, 2017-18. Initial culture of *S. mauritia* was made from larvae collected from rice nurseries of RARS, Warangal, Telangana, India and reared in laboratory (28± 5°C; 65± 5% RH). The egg masses were maintained in 15x 10 cm plastic jars and provided with rice seedlings intact with primary roots as a feed. It was ensured that the seedlings touch the walls of jar by placing them in a test tube so that first instar larvae crawl onto the plant immediately after hatching. Later, these test tubes along with the first instar larvae were transferred to pre-sterilized transparent plastic containers and covered with a muslin cloth. Fresh rice leaves were provided as and when required till the larvae entered the third instar; from this instar onwards, the larvae were released onto the rice seedlings raised in plastic trays containing water, so that the larvae will not escape from the tray. Until the larvae reached the sixth instar, fresh rice seedlings were fed as and when needed. Sixth instar larvae or prepupae were later transferred to soil kept in plastic container for pupation. With the larvae reared in the artificial diet (Hamed and Nadeem, 2007), immediately after hatching the larvae were transferred to petriplates with freshly prepared artificial diet. Pupae obtained were kept in small plastic jars covered with muslin cloth for adult emergence, with the male and female pupae separated by taking into account external characters, until adult emergence. A pair of newly emerged male and female moths were then transferred to new oviposition jar along with blotting paper and fresh leaf for egg laying. The jars were covered with muslin cloth to prevent the escape of the adults. 10% honey solution was provided as food for adults, soaked on a piece of cotton with honey solution. These jars were observed every morning for egg laying. Data on parameters such as preoviposition

and oviposition period, fecundity, incubation, larval and pupal periods, and adult longevity of male and females were recorded. These were analyzed for mean and standard deviation (using one way ANOVA).

RESULTS AND DISCUSSION

The details of survival and developmental stages of *S. mauritia* investigated with artificial diet and rice leaves revealed statistically significant differences (Table. 1); maximum larval period was observed in rice leaves (23.00± 0.52) and using artificial diet (20.00± 0.51). This varied from 21-32 days (Anantanarayanan and Ayyar, 1937) and 38.6 days (Tanada and Beardsley, 1958). The adults reared on artificial diet revealed a longer life span than those reared on rice leaves; egg to adult life span when reared on rice leaves and artificial diet were 38.37± 1.00 and 36.38± 1.00 days, respectively. Eggs were laid in masses of 9-150 eggs covered with greyish anal tuft of hairs of female; eggs laid/ female reared on artificial diet and rice leaves amounted to 1887.50± 21.92 and 1013.50± 21.92, respectively. Female adult longevity was more on both diets compared to that of males; females reared from artificial diet lived for 9.00± 1.00 days and males for 7.00± 1.00 days; with when reared with rice leaves it was 8.00± 1.00 and 6.00± 1.00 days, respectively. No cannibalism was observed among the larvae. Crowding caused cannibalism (Smith, 1933). Thus, the artificial diet was more favourable compared to natural diet of rice leaves. The lifecycle was shortened when larvae were reared on artificial diet; also, number of eggs laid/ female was more. Under the changing climatic conditions, outbreaks of *S. mauritia* may occur at any point of time. The continuous maintenance of laboratory colony is needed for any biochemical and physiological studies, and artificial diets could help in this.

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Table 1. Comparative biology of *Spodoptera mauritia*

S. No.	Stage of the insect	Natural diet Mean (Days)± SD	Range (mm)	Artificial diet Mean (Days)± SD	Range (mm)
1.	Incubation period	3.00± 0.00	3.00-3.00	3.00± 0.00	3.00-3.00
2.	Larval period				
a.	I instar	2.50± 0.52	2.00-3.00	2.00± 0.51	2.00-2.00
b.	II instar	2.50± 0.52	2.00-3.00	2.00± 0.51	2.00-2.00
c.	III instar	3.50± 0.52	3.00-4.00	3.00± 0.51	3.00-3.00
d.	IV instar	3.50± 0.52	3.00-4.00	3.00± 0.51	2.00-4.00
e.	V instar	2.50± 0.52	2.00-3.00	3.00± 0.51	2.00-4.00
f.	VI instar	5.50± 0.52	5.00-6.00	4.00± 0.51	2.50-5.50
	Total larval period	23.00± 0.52	20.0-26.0	20.00± 0.51	18.0-22.0
3.	Pre pupal period	0.88± 0.12	0.75-1.00	0.88± 0.12	0.75-1.00
4.	Pupal period	7.50± 0.52	7.00-8.00	7.50± 0.51	7.00-8.00
5.	Adult longevity				
	Male	6.00± 1.00	5.00-7.00	7.00± 1.00	6.00-8.00
	Female	8.00± 1.00	7.00-9.00	9.00± 1.00	8.00-10.00
	Average	7.00± 1.00	6.00-8.00	8.00± 1.00	7.00-9.00
6.	Total lifecycle	38.37± 1.00	33.75-43.00	36.38± 1.00	31.25-41.50
7.	Preoviposition period	1.75± 0.35	1.50-2.00	1.50± 0.35	1.00-2.00
8.	Oviposition period	3.00± 1.41	2.00-4.00	4.00± 1.41	3.00-5.00
9.	Fecundity (no.)	1013.50± 21.92	998.0-1029.00	1887.50± 21.92	1777.75-1997.25
10.	% hatching	81.50± 8.49	75.50-87.50	87.00± 7.49	82.00-92.00

*Mean of 10 individuals; SD: Standard deviation

AUTHOR CONTRIBUTION STATEMENT

MR conducted survey, identification, rearing, recorded data on biology and manuscript preparation; TUM did planning, guiding, and manuscript preparation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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LIFETABLES OF *CHILO PARTELLUS* (SWINHOE) INFESTING RABI SORGHUM

S K Meena* and V K Bhamare

Department of Agricultural Entomology, College of Agriculture
(Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani 431402), Latur 413512, Maharashtra, India
*Email: sharadkumarmeena01@gmail.com (corresponding author): ORCID-0000-0002-2824-3739

ABSTRACT

This study on the sorghum spotted stem borer *Chilo partellus* (Swinhoe) was conducted at the Post Graduate Experimental Field of Department of Agricultural Entomology, College of Agriculture, Campus Latur during 2020-21. The results revealed that *C. partellus* passed through three generations on rabi sorghum. The mortality in early instar larval stage was observed due to unknown reasons (17.24, 19.05 and 14.51%, respectively), *Callibracon* sp. (4.16, 11.76 and 8.33%, respectively) and *Cotesia flavipes* (8.69, 6.67 and 9.65%, respectively) in its first, second and third generations. The mortality in late instar larvae was also found owing to unknown reasons (19.4, 14.28, and 11.36%, respectively), *Callibracon* sp. (11.76, 12.49 and 11.43%, respectively) and *C. flavipes* (13.33, 14.28 and 10.26%, respectively) in the first, second and third generations. In first generation, the pupal mortality was not observed, and when noticed it was due to unknown reasons (11.11 and 16.12%, respectively) during second and third generations. The trend index and generation survival were 1.44 and 0.44; 1.48 and 0.38 and; 0 and 0.42 during first, second and third generations, respectively.

Key words: *Chilo partellus*, rabi sorghum, lifetable, mortality, unknown reasons, instars, pupal stage, *Callibracon* sp., *Cotesia flavipes*, parasitoid, trend index, generation survival

Sorghum (*Sorghum bicolour* (L.) Moench) is a warm-season cereal of African origin, and it is ravaged by a number of insect pests viz., shoot fly (*Atherigona soccata* Rondani), stem borers [*Chilo partellus* (Swinhoe) and *Sesamia inferens* Walker], army worms (*Mythimna separata* Walker and *Spodoptera frugiperda* J E Smith), aphids (*Melanaphis sacchari* Zehntner and *Rhopalosiphum maidis* Fitch), midge (*Contarinia sorghicola* Coquillett), earhead caterpillars (*Helicoverpa armigera* Hubner), hairy caterpillars (*Orgyia* sp., *Olene mendosa* Hubner and *Somena scintillans* Walker), shoot bugs (*Peregrinus maidis* Ashmead) and green stink bug (*Nezara viridula* (L.) in Maharashtra. In sorghum fields, >35% crop losses have been reported due to insect pests, estimated to be at $580 million in India (Reddy and Zehr, 2004). In India, *C. partellus* (Swinhoe) (Crambidae: Lepidoptera) is one of the serious insect pests causing 24.3 to 36.3% yield loss (Kaur et al*.,* 2020). The present study explored the fluctuations in the population dynamics through the lifetables for understanding the mortality factors of *C. partellus* on rabi sorghum. This might b helpful to develop the IPM strategies and identify various natural enemies. Study on lifetable is required to understand the influence of abiotic and biotic factors at different life stages (Pathaek and Bhamare, 2019).

MATERIALS AND METHODS

The field experiment comprising forty-eight quadrats each of 2.70 x 3.00 m size was laid out with rabi sorghum at the Research Farm of Department of Agricultural Entomology, College of Agriculture, Latur (MS) during rabi 2020-2021.The popular variety Parbhani Moti was sown at the spacing of 45 x 15 cm in 48 quadrats following recommended package of practices by VNMKV, Parbhani. The field experiment was conducted under pesticide free conditions. The sampling of eggs, early and late instar larvae and pupae of *C. partellus* was done on the basis of development in laboratory reared culture. At each observation, three quadrats of sorghum were carefully examined twice in a week for the number of eggs, larvae and pupae. The field collected eggs, larvae and pupae were brought to the laboratory and reared on sorghum plant parts in plastic vials (measuring 5 cm height and 4 cm dia) and boxes (measuring 15x 20 cm). The food was changed as and when required until adult emergence. The observations were made on the egg, larval and pupal parasitism as well as mortality because of unknown reasons and entomopathogens in early and late larval instars and pupal stage. An interval of four to six days was provided before sampling of next generation after the mean adult

emergence of previous generation. This period was considered for completion of act of oviposition by the moth of previous generation. The newly hatched first instar larvae were collected in subsequent generations.

The lifetable was constructed based on Morris and Miller (1954) and Harcourt (1969)/ X= age interval, egg, larva, pupa and adult; lx = number surviving at the beginning of stage noted in 'x' column; dx = number dying within the age interval stated in 'x' column; dxF = mortality factor responsible for 'dx'; 100qx = % mortality; and Sx= survival rate within the age mentioned in 'x'column. The trend index was simply 'lx' for the early instar larvae in the next generation expressed as a ratio of previous generation. It was calculated with the formula N2 / N1 were N2 is equal to the population of early instar larvae in next generation and N1 is equal to the population of early instar larvae in previous generation. The generation survival was an index of population trend without the effect of fecundity and adult mortality; it calculated with the formula N3/ N1- where N3 is equal to population of adult in a generation and N1 is equal to population of early instar larvae in the same generation. A separate budget was prepared to find out the key factors responsible for the changes in the population trend of *C. partellus* on sorghum. The method of key factors analysis developed by Varley and Gradwell (1963; 1965) was used to detect density relationship of mortality factors. By this method, the killing power (K) of such mortality factors or group of mortality factors in each age group was estimated as the difference between the logarithms of population density of the killing power of 'k's.

RESULTS AND DISCUSSION

Chilo partellus completed three regular overlapping generations on rabi sorghum. The results on lifecycle and key mortality factors in 1st, 2nd, and 3rd generation are presented in Table 1 and Fig. 1-3. The mortality in early instar larval stage was- due to unknown reasons (17.24, 19.05 and 14.51%, respectively); *Callibracon* sp. (4.16, 11.76 and 8.33%, respectively); and *Cotesia flavipes* (8.69, 6.67 and 9.65%, respectively) in first, second and third generations. In the late instar it was- due to unknown reasons (19.4, 14.28, and 11.36%, respectively); *Callibracon* sp. (11.76, 12.49 and 11.43%, respectively); and *C. flavipes* (13.33, 14.28 and 10.26%, respectively) in first, second and third generations. In first generation the pupal mortality was not found, while it was- due to unknown reasons (11.11 and 16.12%, respectively) during second and third generations. The trend index and generation survival were- 1.44 and 0.44;

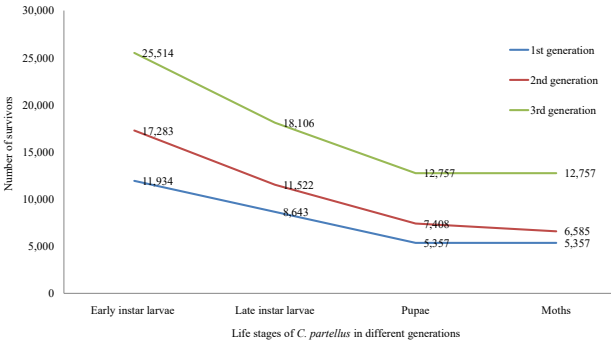

Fig. 1. Survivorship curve of different generations of *C. partellus* sorghum during *rabi* season 2020-21

1.48 and 0.38 and; 0 and 0.42 during first, second and third generations, respectively. The maximum generation mortality during first, second and third generations was noticed from late instar, early instar and late instar larvae (k= 0.221 and k=0.152, respectively). Total K for first, second, and third generation was 0.649, 0.719, and 0.679, respectively (Table 1).

The above results agree with those of Singh et al. (2020) who documented that maximum larval parasitisation of *C. partellus* was recorded by *C. flavipes* (31.64%). Kaur et al. (2020) observed that larvae of *C. partellus* were parasitised (28.6-100, 41.4-50 and 20-80%) by *C. flavipes*. The result indicated that parasitism by *Cotesia* was influenced by age of plants. Hassan et al. (2020) revealed that larval parasitism due to *C. ruficrus* was in the range of 9.77-22.22%; while Dejen et al. (2020) indicated that *C. flavipes* caused less parasitism on stem borers in maize compared to sorghum; *C. flavipes* caused 82% parasitism on *C. partellus.* Rai and Prasad (2019) revealed that *C. flavipes* was the dominant natural enemy with maximum parasitisation of 57%. Sokame et al. (2019) observed that maize stem residues had a higher abundance of *C. flavipes* and *C. sesamiae* parasitoids than wild plants. Kumar (2019) revealed that the major mortality factors of *C. partellus* were the larval parasitoids particularly *C. flavipes* (21.60 to 47%) and unknown causes during early and middle larval stages.

The trend index was positive (>1) and varied in all the generations. Suneel Kumar et al. (2018) observed a peak parasitism of *C. partellus* by *C. flavipes* during 40th SMW in kharif and 4th SMW in rabi. Kumar (2017) revealed that the larval mortality was 37 and 16.07% due to parasitisation and unknown factors, respectively; and mortality of pupae was 11.76% due to diseases while 15.38% failed to emerge into moths. The total mortality (K value) of *C. partellus* was 0.88 due to the effect of biotic and abiotic factors. Patel et al. (2012) showed that *Apanteles* was active from third week of

Table 1. Field lifetable and budget generations of *C. partellus* on sorghum (rabi, 2020-21)

Age interval	No. alive/ ha at the beginning of x	Factors responsible for d_x	No. dying during x	dx as % of l_x	Survivalrate at age X
X	l_x	d_xF	d_x	$100q_x$	S_x
First generation					
Early instar larvae (N_1)	11,934	Unknown reasons	2,057	17.24	0.72
	9,877	*Callibracon* sp.	411	4.16	
	9,466	*Cotesia flavipes*	823	8.69	
Late instar larvae	8,643	Unknown reasons	1,646	19.04	0.62
	6,997	*Callibracon* sp.	823	11.76	
	6,174	*C. flavipes*	823	13.33	
Pupae	5,357	-	-	-	1.00
Moths	5,357	Sex 50% Females	-	-	-
Females x 2 (N_3)	2,675	(Reproducing females=2,675)	-	-	-
Trend index (N_2/ N_1)	17,228 / 11,934	-	1.44	-	-
Generation survival (N_3/ N_1)	5,357 / 11,934	-	0.44	-	-
Second generation					
Early instar larvae (N_1)	17,283	Unknown reasons	3,292	19.05	0.67
	13,991	*Callibracon* sp.	1,646	11.76	
	12,345	*C. flavipes*	823	6.67	
Late instar larvae	11,522	Unknown reasons	1,646	14.28	0.64
	9,876	*Callibracon* sp.	1,234	12.49	
	8,642	*C.flavipes*	1,234	14.28	
Pupae	7,408	Unknown reasons	823	11.11	0.88
Moths	6,585	Sex 50% Females	-	-	-
Females x2 (N_3)	3,292	(Reproducing females=3,292)	-	-	-
Trend index (N_2/ N_1)	25,514 / 17,283	-	1.48	-	-
Generation survival (N_3/N_1)	6,585 / 17,283	-	0.38	-	-
Third generation					
Early instarlarvae (N_1)	25,514	Unknown reasons	3,703	14.51	0.71
	21,311	*C. flavipes, Callibracon*	2,057	9.65	
	19,754	sp.	1,646	8.33	
Late instarlarvae	18,106	Unknown reasons	2,057	11.36	0.70
	16,049	*C.flavipes, Callibracon*	1,646	10.26	
	14,403	sp.	1,646	11.43	
Pupae	12,757	Unknown reasons	2,057	16.12	0.83
Moths	12,757	Sex 50% Females	-	-	-
Females x2 (N_3)	5,350	(Reproducing females=5,350)	-	-	-
Trend index (N_2/N_1)	0 / 25,514	-	0	-	-
Generation survival (N_3/N_1)	10,700 / 25,514	-	0.42	-	-

S. No.	Age interval	'k' values of generations of *C. partellus*		
		1st	2nd	3rd
1.	Early instar larva	-	-	-
2.	Late instar larva	0.140	0.221	0.149
3.	Pupa	0.208	0.146	0.152
4.	Adults	0.000	0.051	0.077
5.	Reproducing females	0.301	0.301	0.301
	Total 'K'	0.649	0.719	0.679

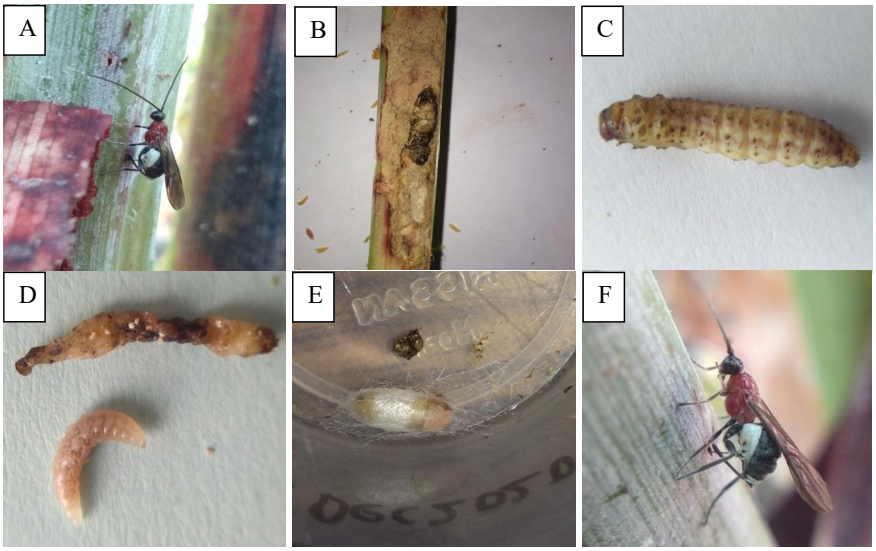

Fig. 2. Parasitisation of *C. partellus* larva by *Callibracon* sp. A. Parasitisation by *Callibracon* sp B. Parasitised larva in stem C. Parasitised larva D. Grub of *Callibracon* sp. E. Cocoon of *Callibracon* sp. F. Adult of *Callibracon* sp.

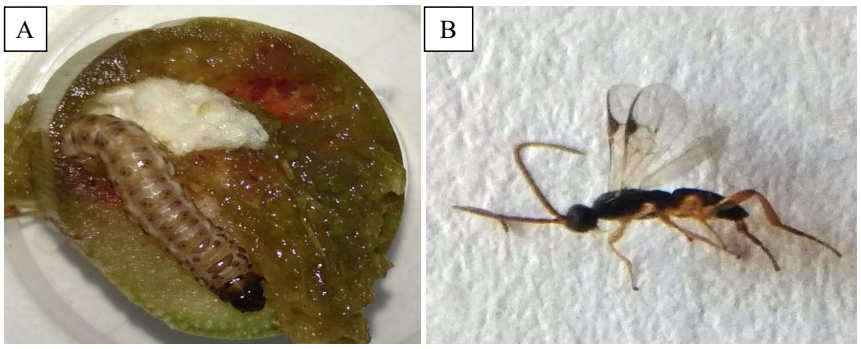

Fig. 3. Parasitisation of *C. partellus* larva by *Cotesia flavipes*. A. Parasitised larva of *C. partellus* and cocoons of parasite B. Adult of *C. flavipes*

August to first week of November. Divya et al. (2009) revealed maximum parasitisation by *C. flavipes* during 45th SMW (35%) during kharif and in 50th SMW (32%) during rabi-summer. Midega et al. (2005) observed that mortality by *Cotesia sesamiae* Cameron and *C. flavipes* was very minimal. Jalali and Singh (2003) reported that larval parasitoid, *Cotesia flavipes* (Cameron) was very important. Jalali et al. (2003) illustrated that larval parasitoids viz., *C. flavipes*, *Myosoma chinensis* (Szepligeti) and *Stenobracon nicevellei* (Bingham) and insect pathogens were insignificant factors in the mortality of *C. partellus*.

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JOINT ACTION OF BOTANICALS AGAINST *HELOPELTIS THEIVORA* WATERHOUSE AND *OLIGONYCHUS COFFEAE* NIETNER

Priyanka Borbaruah* and Surajit Kalita**

*Department of Entomology; **Directorate of Research (Agri),
Assam Agricultural University, Jorhat 785013, Assam, India
*Email: priyanka.borbaruah.adj20@aau.ac.in (corresponding author)

ABSTRACT

Laboratory experiments to evaluate the combined effect of *Chromolaena odorata (Co)* and *Phlogocanthus thyrsiflorus (Pt)* against *Oligonychus coffeae* and *Helopeltis theivora* revealed that the methanolic leaf extracts at a combination of 75% LC_{50} *Pt* + 25% LC_{50} *Co* and 25% LC_{50} *Pt* + 75% LC_{50} *Co* were the most effective against *O. coffeae* and *H. theivora*, respectively. They recorded the highest co-toxicity coefficient and co-toxicity factor values of 206.53 and 63.34 and 398.40 and 100.00, respectively revealing potentiation and synergistic effects. A combination of 50% LC_{50} *Pt* + 50% LC_{50} *Co* can effectively manage both *O. coffeae* and *H. theivora* (76.67% and 100.00% adult mortality, respectively) with the highest co-toxicity coefficient and co-toxicity factors.

Key words: *Chromolaena odorata, Phlogocanthus thyrsiflorus,* leaf extract, LC_{50}, bioefficacy, adult mortality, *Helopeltis theivora, Oligonychus coffeae*, joint action, co-toxicity

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most popular, and widely consumed non-alcoholic beverage (Kumar and Shruthi, 2014). India is the largest producer and consumer of black tea (Soni et al., 2015). Tea is an evergreen, perennial, monocultured and commercial crop (Wight,1961). Tea harbours about 1031 species of arthropod pests (Hazarika et al., 2009a) including 250 insects (Barthakur, 2011). Mirids are the major insect pests of tea in Asian countries including India causing about 11 to 100% loss (Hazarika et al., 2009a). Out of 41 described species of mirids belonging to the genus *Helopeltis* in Asia, the tea mosquito bug (TMB) *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) is the major pest in Assam (Somchowdhury et al., 1993). Spider mites are also a serious and persistent pest of tea resulting a crop loss of 17-46%. In India, the red spider mite (RSM) *Oligonychus coffeae* Nietner (Acarina: Tetranychidae) is the most important reported from Assam in 1868 (Hazarika et al*.,* 2009b). To defend the tea crop from insect pests, pesticides are used resulting in resurgence of primary pests, secondary pest outbreak, development of insecticide resistance, and environmental contamination, including undesirable residue (Hazarika et al., 2009a; Sannigrahi and Talukdar, 2003). In contrast, botanicals are an important alternative to pesticides. *Chromolaena odorata* (L.) King & Robinson (Asteraceae) and *Phlogocanthus thyrsiflorus* Nees (Acanthaceae) are the most widely distributed and naturally grown perennial shrub of North Eastern India, which to contain essential oils, antimicrobial flavonoids, alkaloids, fatty acids and phenolic compounds with antioxidant, anti-inflammatory, antifungal, antimicrobial and antiradical properties (Lawal et al., 2015). This study focused on the bioefficacy of solvent extracts of *P. thyrsiflorus* and *C. odorata,* which revealed a strong acaricidal and insecticidal property, respectively (Borbaruah and Kalita, 2019; 2020).

MATERIALS AND METHODS

Mature leaves of *P. thyrsiflorus* and *C. odorata* were collected from their natural habitats of Borbheta and Lichubari areas of Jorhat, Assam (26.746°N, 94.2026°E) during September-October, 2017, shade-dried, powdered using an electric grinder and extracted with methanol using Soxhlet apparatus (Make: Labotech) (Borbaruah and Kalita, 2019; 2020). The methanolic leaf extracts were later dried *in vacuo* and the supernatant was dissolved in equal volume of acetone on w/v basis to make 100% stock solution. The stock solution was then stored in an airtight glass bottle at 4ºC for further use. The mass rearing of the target pests was done at the Physiology Laboratory, Department of Entomology, Assam Agricultural University, Jorhat. The adults of *O. coffeae* and *H. theivora* were collected from the Experimental Garden for Plantation Crops (EGPC), Assam Agricultural University, Jorhat and cultured on detached leaves following the detached leaf technique suggested by Das et al. (2012; 2017). Twenty one-day-old laboratory cultured *O. coffeae* adults were placed on a TV1 clone leaf disc of 2.5 cm^2 area and allowed to

settle for 24 hr (Borbaruah and Kalita 2019; 2020). The leaf discs containing mites were then sprayed with plant extracts at desired concentrations using a hand atomizer (Make: Axiva, Capacity: 50 ml) (Borbaruah and Kalita, 2019; 2020) and data on morality were recorded at 6, 12, 24 and 48 hr after treatment (HAT).

To study the bioefficacy of plant extracts against *H. theivora*, three freshly detached shoots (TV1 clone) with three leaves were collected from EGPC, AAU, wrapped with absorbent cotton and placed in a glass vial (7 x 3.5 cm dia), and the vials were filled with sterilized double distilled water to keep the shoots afresh for a longer period. The glass vials were then caged with hurricane lantern glass chimney covered with muslin cloth to prevent the escape of the insect (Borbaruah and Kalita, 2019; 2020). A total of five one-day aged old adults were then released and sprayed with plant extracts at desired concentrations with a hand atomizer. Treatments were replicated thrice and the data on adult mortality recorded at 6, 12, 24 and 48 HAT. The data on adult mortality was corrected with Abbott's formula (Abbott, 1925) and subjected to angular transformation for ANOVA. The data on corrected mortality was later subjected to the probit analysis for calculation of LC_{50} values using SPSS (ver. 12.0). The joint action analysis of *P. thyrsiflorus* and *C. odorata* against *O. coffeae* and *H. theivora* was carried out with treatment combinations and the co-toxicity coefficient was calculated following Sun and Johnson (1960). When the treatment combination gives a coefficient significantly greater than 100, it indicates synergistic action.

RESULTS AND DISCUSSION

The methanolic leaf extract of *P. thyrsiflorus* was found to have strong acaricidal properties and recorded the lowest LC_{50} value of 0.531% (R=0.05, χ^2= 5.09, Y= 0.36+1.32X) against *O. coffeae* at 48 HAT, while the LC_{50} value against *H. theivora* was recorded to be 28.348% (R=0.11, χ^2= 9.57, Y= -1.75+1.21X) at 48 HAT. Previous studies had also revealed strong pesticidal properties of the methanolic leaf extract against *O. coffeae as* compared to the water extract (Bora et al., 1999; Hazarika et al., 2000). Mech et al. (2015) reported the lowest LC_{50} values of 0.12% against *O. coffeae* on application of the methanolic leaf extract of *Parthenium hysterophorus*. Roy and Gurusubhramanian (2011) reported strong pesticidal properties of commercial neem formulations against *H. theivora* with lower LC_{50} values ranging between 0.16-2.27 ppm. A strong acaricidal property of *P. tubiflorus* was also reported by Hazarika et al. (2009b) causing reduction in fecundity of *O. coffeae*. The strong acaricidal properties of *P. thyrsiflorus* might be due to the presence of β- sitosterol, lupeol, betulin, phloganthoside and phlogantholideon (Ilham et al., 2012). The methanolic leaf extracts of *C. odorata* revealed a strong pesticidal property recording the lowest LC_{50} value of 0.056% (R=0.25, χ^2= 28.24, Y= 3.48+2.81X) against *H. theivora* at 48 HAT. The LC_{50} value of the methanolic leaf extract of *C. odorata* against *O. coffeae* was found to be 0.603% (R=0.05, χ^2= 38.87, Y= 0.28+1.30X) at 48 HAT. A similar kind of strong insecticidal property was reported in the case of *C. odorata* against *Aedesa egypti* (Sukhthankar et al*.,* 2014; Rajmohan and Logankumar, 2011), which might be due to the presence of alkaloids and flavonoids (Man, 2013; Acero, 2014). The lowest LC_{50} values of *C. odorata* and *P. thyrsiflorus* recorded against *O. coffeae* and *H. theivora* were taken into consideration for the joint action analysis.

The joint action analysis of the methanolic leaf extract of *P. thyrsiflorus* and *C. odorata* with different treatment combinations against *O. coffeae* and *H. theivora* revealed the highest of 81.67% adult mortality of *O. coffeae* in the case of T2 (75% LC_{50} *Pt* + 25% LC_{50} *Co*) at 48 HAT, while the treatment combinations *viz*., T3 (50% LC_{50} *Pt* + 50% LC_{50} *Co*) and T4 (25% LC_{50} *Pt* + 75% LC_{50} *Co*) was found to be the best recording 100.00% adult mortality of *H. theivora* at 48 HAT. The joint action analysis based on calculated co-toxicity coefficient revealed *C. odorata* and *P. thyrsiflorus* to be synergistic with probability of similar action. The co-toxicity coefficient (206.53) was reported to be the highest in the treatment T2 (75% LC_{50} *Pt* + 25% LC_{50} *Co*) against *O. coffeae*. The highest co-toxicity coefficient value of 398.40 was recorded in the treatment T4 (25% LC_{50} *Pt* + 75% LC_{50} *Co*) against *H. theivora* (Table 1). The treatment combination T3 (50% LC_{50} *Pt* + 50% LC_{50} *Co*) could be considered as the most prospective one, which recorded the highest adult mortality of 76.67% and 100.00% against *O. coffeae* and *H. theivora* with a higher co-toxicity coefficient of 200.00.

Botanical is considered as an integral part of any ecofriendly management practices to overcome the ill effect of synthetic pesticides, especially the pesticide residue on the made tea. The present study has identified a suitable combination of *C. odorata* and *P. thyrsiflorus* leaf extract that could manage the *H. theivora* and *O. coffeae* with a single spray.

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Table 1. Joint action analysis of *C. odorata* and *P. thyrsiflorus* against *O. coffeae* and *H. theivora*

Treatments	*O. coffeae*			*H. theivora*		
	Adult mortality (%)		Co- toxicity coefficient	Adult mortality (%)		Co-toxicity coefficient
	Expected	Observed		Expected	Observed	
T1 (100% LC_{50} *Pt** + 0% LC_{50} *Co***)	50	53.33	_	50	46.67	_
T2 (75% LC_{50} *Pt* + 25% LC_{50} *Co*)	50	81.67	206.53	50	80.00	133.51
T3 (50% LC_{50} *Pt*+ 50% LC_{50} *Co*)	50	76.67	200.00	50	100.00	200.00
T4 (25% LC_{50} *Pt* + 75% LC_{50} *Co*)	50	56.67	193.90	50	100.00	398.40
T5 (0% LC_{50} *Pt* + 100% LC_{50} *Co*)	50	43.33	_	50	60.00	_

*LC_{50} value of *P. thyrsiflorus* considered against *O. coffeae*= 0.531%, **LC_{50} value of *C. odorata* considered against *H. theivora*= 0.056%

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NEW RECORD OF *LUCILIA SERICATA* (WIEDEMANN) FROM KARGIL LADAKH

Mohd Hussain*, Altaf Hussain Mir[1], Hidayatullah Tak [1] and Nassreen F Kacho[2]

Department of Zoology, Leh campus, University of Ladakh 194101, Ladakh (UT), India
[1]Department of Zoology, Kashmir University Srinagar 190006, Jammu and Kashmir (UT), India
[2]KVK1, SKUAST-K, Kargil Ladakh 190025, Ladakh (UT), India
*Email: akmha168@gmail.com (corresponding author)

ABSTRACT

***Lucilia* spp. also known as green bottle fly is well distributed, and it has forensic, medical and veterinary importance. In the present study, *Lucilia sericata* (Wiedemann, 1830) has been recorded from the Trans-Himalayan region of the cold arid desert Kargil Ladakh. Distribution over a period from April 2018 to March 2021 revealed that it is fairly distributed with maximum abundance being in midsummer July and August, with no activities during November to March.**

Key words: *Lucilia sericata*, Trans-Himalaya, new record, abundance, occurrence, diagnosis, illustrations

Lucilia spp. is commonly known as green bottle fly and always found in human inhibited areas including slaughterhouses, meat shops, latrines and waste dumping places from where they possibly transmitt many pathogens including nematodes, helminths, protozoans, fungus, bacteria and viruses to human and other vertebrates (Fetene and Worku, 2009; Khoobdel et al., 2013; Akbarzadeh et al., 2015; Hasson, 2017; Tomberlin et al., 2017). This species is almost cosmopolitan in distribution and have forensic, medical and veterinary importance (Nandi, 2002). As per the literature survey, only few species of the Calliphoridae family viz. *Calliphora vicina, C. vomitoria* and *Protophormia terraenovae* have been recorded from the Trans-Himalayan region of the cold arid desert Ladakh and no record were found related to the *Lucilia* sp. (Hussain et al., 2021a, Hussain et al., 2021b). The present study recorded *L. sericata*, their distribution and seasonal abundance from the Kargil Ladakh.

MATERIALS AND METHODS

The present study was conducted in the trans-Himalayan region of the cold arid desert Kargil Ladakh (30-35"N,75-77"E- LAHDC-Kargil, 2022). Monthly (11- 20th) surveys were conducted from April 2018 to March 2021, dividing it into eight main sites viz. Drass, Kargil town, Batalik, Chiktan, Wakha (Shargole), Trespone, Sankoo and Panikhar. Plastic bottle traps baited with 100 g unwashed goat/ sheep stomach were used (Hussain et al., 2021a,b). On each visit, three traps were installed at a distance of about 100 m for three hours extending from 11:00 am to 02:00 pm; it was around the places like slaughterhouses, local latrine, meat shops and waste dumping areas. The trapped flies were killed using chloroform, and based on their morphology *Lucilia* spp. were sorted out, counted and identified up to species level using available keys (Crosskey and Lane, 1993; Wallman, 2001; Whitworth, 2006; Carvalho and Mello-Patiu, 2008). Photographs were captured by using Leica S9i stereozoom microscope fitted with camera and developed with Adobe Photoshop 7.0. Data were analyzed using software SPSS 16.0 and graphs were plotted by using software Origin pro 8. Weather data of the district Kargil were obtained from the Indian Metrological Department, Meteorological Centre, Rambagh, Srinagar, Jammu & Kashmir UT, India.

RESULTS AND DISCUSSION

Lucilia sericata (Wiedemann, 1830)

Diagnosis: Body metallic green in colour; parafrontalia broad and white; frontoclypeal membrane light brown; width of frontal stripe (frontal vitta) twice

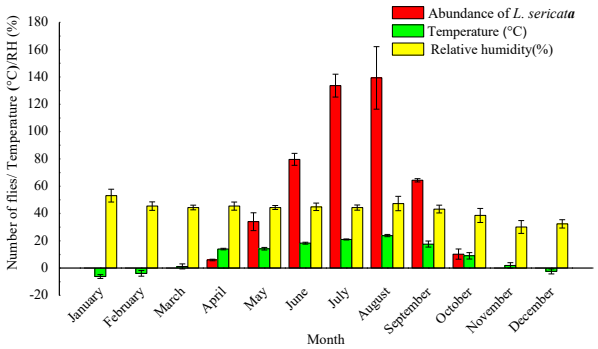
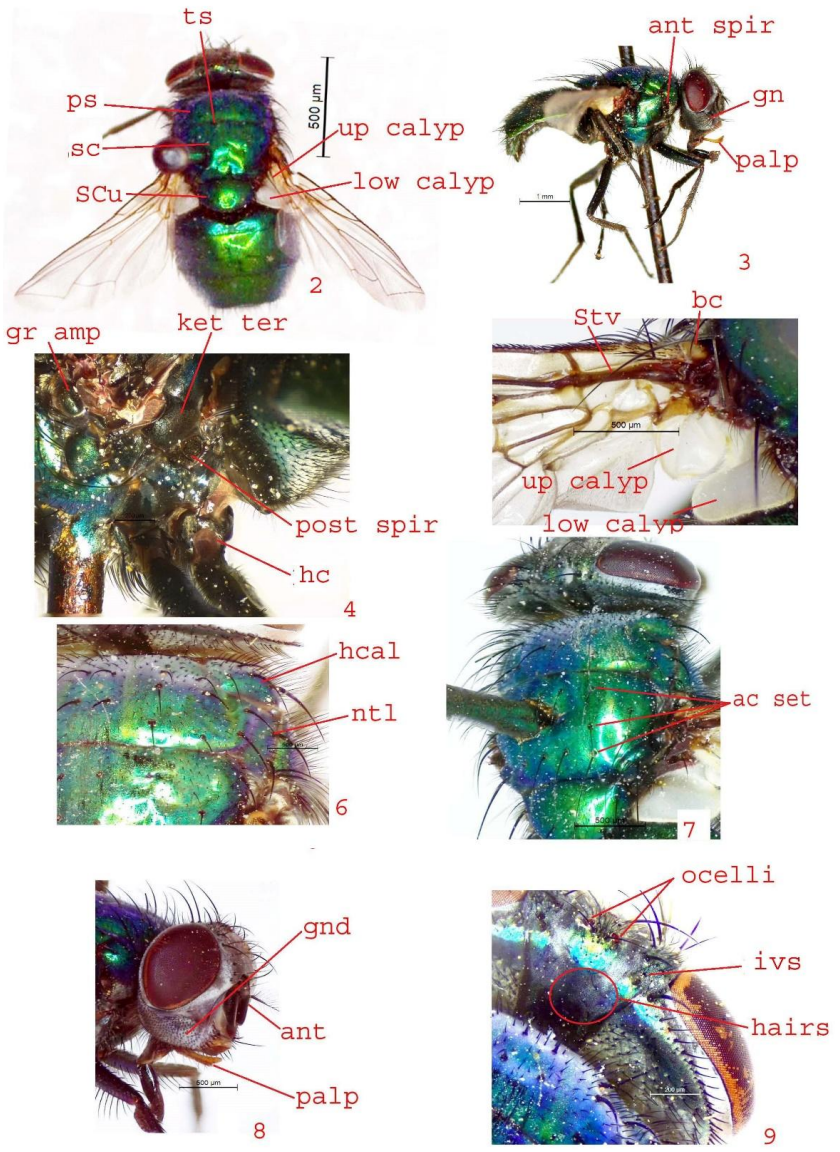


Fig. 1. Seasonal abundance of *L. sericata*- Kargil Ladakh (April 2018- March 2021)

as wide as parafrontal plate; gena white with black hairs; Humeral callus with 6-8 hairs on posterior slope; notopleuron with 8-16 hairs; central occipital area with 2-8 setulae below each inner vertical seta; Ketatergite bar; wings hyaline; basicostae bright yellow; lower calytpter without hairs above; stem vein bar above; colour of the fore femora dark metallic blue to black or brown (Figs. 2-9).

Distribution and abundance: It was observed that *L. sericata* was fairly distributed in the study area, and 1412 specimens were captured from April 2018 to March 2021; of these Kargil town contributed maximum of 244 (17.28%) followed by Sankoo 197 (13.95%), Chiktan 181 (12.82%), Batalik 170 (12.04%), Panikhar 163 (11.54%), Drass 160 (11.33%), Trespone 156 (11.05%) and Wakha 141 (9.99%). Kargil town is a densely populated area and have many slaughterhouses, meat shops chicken shop in the main market and in surrounding areas without proper waste management



Figs. 2-9. *Lucilia sericata*; 2. Whole body, dorsal view; 3. Whole body, lateral view; 4. Lower part of posterior thorax, lateral view; 5. Wing and calypters, dorsal view; 6 and 7. Thorax, dorsal view; 8. Head, lateral view; 9. Head, dorsal view

Abbreviations used: ts = transverse suture; ps = prescutum; sc = scutum; SCu = scutellum; up calyp = upper calypter; low calyp = lower calypter; ant spir = anterior spiracle; gn = gena; gnd = genial dilation; post spir = posterior spiracle; gr amp = greater ampulla; ter = ketatergite; hc = hind coxa; bc = basicosta; stv = stem vein; h cal = humeral callus; ntl = notopleuron; ant = antenna; ac set = acrostichal bristles; ivs = inner vertical setae.

(LAHDC-Kargil, 2022). It provides a breeding ground and with maximum abundance as given in Brundage et al. (2011). Its abundance is affected by weather factors as shown by earlier workers (Hwang and Turner, 2005; Hussain et al., 2021a); it exhibited similar conditions as with *C. vicina* (Hussain et al., 2021a); *L. sericata* began its activity in April and increased with temperature reaching a maximum in July (133.66± 8.37) and August (139.33± 22.92), and declined with decrease in temperature from September and becoming nil in winter (Fig. 1). Its abundance revealed strong positive correlation with temperature (r=0.88) and a weak positive one with relative humidity (r=0.285). These results corroborate with those of Brundage et al. (2011).

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COMPOSITION OF MOSQUITO SPECIES IN THREE SELECTED VILLAGES IN MAHABUBNAGAR DISTRICT, TELANGANA

Shekhar Mandla* and Suryakala Illendula

Department of zoology, Faculty of Sciences, Osmania University, Hyderabad 500007, Telangana, India
*Email: shekhar20zoo@gmail.com (corresponding author)

ABSTRACT

Mosquito prevalence was studied in three areas of Mahabubnagar district of Telangana viz. Mannanur, Janampeta, B Veerapur. Mosquito sampling was done in three selected villages in Mahabubnagar district in all seasons from 2014-2016. Density, Distribution and habitat preferences are studied for three years 2014- 2016. A total of 1787 mosquitoes were collected. Identification was done using identification keys of Christophers (1933) and Barraud (1934). Three genera *Culex*, *Aedes* and *Anopheles* were observed. *Anopheles* contributes 44%, *Culex* 39% and *Aedes* contributes 17%. *Culex quinquefasciatus* is predominant species (16%), while *Aedes albopictus* (15%) and *Culex gelidus* (15%) occupy the second place. *Anopheles culicifacies* contributes 12%, while *Culex tritaeniorhynchus* (8%) and *Anopheles vagus* (8%) contribute equally. *Anopheles subpictus* (7%), and *Anopheles stephensi* (7%) contributed equally, while *Aedes aegypti* is with only 5%. Human dwellings inhabit 50% due to breeding sites; cattle shed have 25%, and rain water ditch have very less, as these are available only in monsoons. *Cx. quinquefasciatus*, *Cx. gelidus* were found in all habitats, while *An. subpictus*, *An. stephensi*, *An. culicifacies*, *Ae. aegypti*, *Ae. albopictus* were found in some of the habitats.

Key words: Mosquito diversity, vectors, habitat preference, predominant species, breeding site, seasonal variation, *Culex*, *Aedes* and *Anopheles*

Identifying mosquitoes properly and managing their population levels has great impact on their control. Today mosquitoes are creating a great havoc in lives of mankind by acting as carrier for many diseases. Mosquitoes act as vectors for the transmission of viruses, protozoa, bacteria etc. and causing diseases like malaria, dengue, chikungunya, dengue haemorrhagic fever, filariasis, west nile virus fever, yellow fever, encephalitis, etc. Mosquitoes are adaptable insects which continue to co-exist with man and transmit many diseases. Annually 2 million people die due to these mosquito-borne diseases while morbidity rates are still higher many times. (WHO, 2009). Mosquitoes breeds in almost all types of lentic bodies. The mosquito population density in an area is one of the major factors responsible for transmission of diseases in a particular area. The efficiency of any vector control method adopted needs a complete understanding of vectors residing in an area and process of seasonal fluctuations in population density under natural conditions. It will help to undertake a more strategic approach to control and implement various economical, efficient and effective mosquito control program. In India, a study on fauna of mosquito species in various areas has already been conducted. This study was carried out to know the mosquito species composition and their relative abundance in Mahabubnagar, Telangana. The purpose of this work is to study abundance, distribution, diversity, and potential vectors of diseases throughout the study sies.

MATERIALS AND METHODS

Mahabubnagar district, Telengana (18.1124°N, 79.0193°E) has an area of 2737.96 square kilometers and a population of 919903 (2011 census). Three villages in Mahabub Nagar district are chosen for collection of mosquitoes. In Mahabubnagar, 1) Janampeta of Pebbair Mandal, 2) B. Veerapuram of Itikyal Mandal and 3) Mannanur of Amrabad Mandal are chosen. Janampeta is equipped with well-organized irrigation system, and also two big ponds surround the village. B Veerapuram is very adjacent to river Krishna, and is very small village. Mannanur is an agency area, located in the dense forest range of Nallamalla belt. Mosquitoes were sampled every fortnight, all seasons viz; summer, monsoon, winter from 2014 to 2016. Mouth aspirator and sweep net were used for adult collection, and ladle and sweep net used for immatures. Collection was done around 5:30 to 7 am, and 5:30 to 7 pm. Each mosquito was identified based on morphological keys by hand lens in the field and by binocular and

stereozoom microscope in the laboratory based on keys of Christophers (1933) and Barraud (1934).

RESULTS AND DISCUSSION

A total of 1787 mosquitoes collected of the genera *Aedes, Anopheles* and *Culex* from Mahabubnagar district, out of which *Anopheles* contributes 44%, *Culex* 39% and *Aedes* contributes 17% with nine species in all. *Culex gelidus* (20%) is predominant; *Anopheles subpictus* (15%) occupies the second place (Fig. 1). Among the habitats human dwellings are the most preferred one (50%) More mosquitoes were captured at cattle shed (25%); with cement tanks preferred by less number of mosquitoes (Fig. 2). *Culex quinquifasciatus* found in all types of habitats; *C. gelidus* also similar with high number found at cattle shed. *Anopheles subpictus* is found in mud pot, human dwellings and cattle shed, most number is collected at cattle shed. *Anopheles stephensi* is found more at human dwellings. *Aedes aegypti* numbers are less. *Culex tritaniorhychus* found in all except rain water ditch habitat. *Aedes albopictus* is collected high in cement tank, not found in mud pot and cattle shed. *Anopheles culicifacies* reported in high numbers at human dwellings and not found in rain water ditch and cattle shed. Among the species *Anopheles culicifacies* most dominant followed by *Culex gelidus*. *An. subpictus*, *Cx. quinquifasciatus*, *Cx. gelidus* found in sites, showed 100% distribution, rest of the species show 30% distribution. Mannanur site

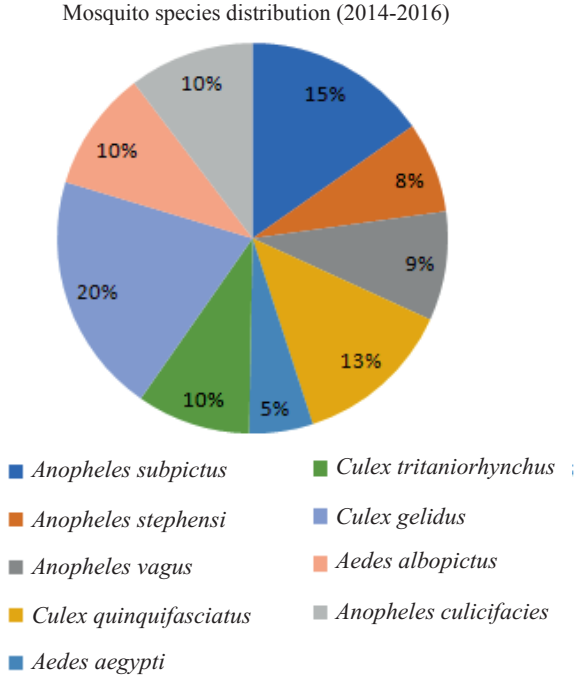

Fig. 1. Mosquito species distribution (2014-16)

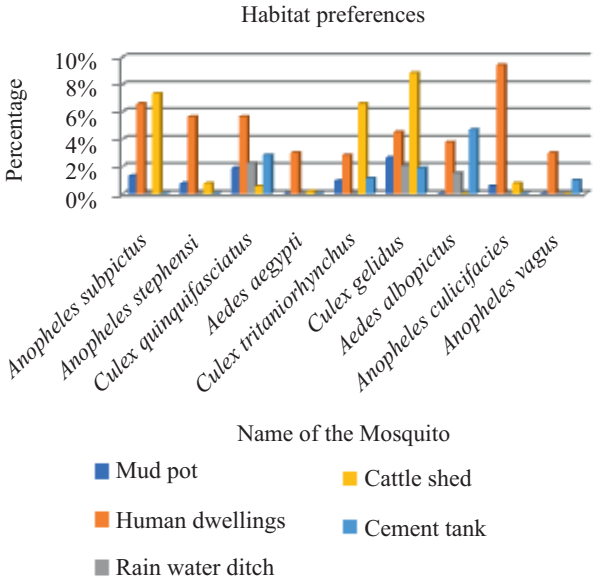

Fig. 2. Showing habitat preferences by mosquitoes

was with *Anopheles subpictus* high in 2016, compared to 2015 and 2016; and was the dominant one. tage to the population. *Anopheles stephensi* populations shown upward trend i.e. increased from 2014-2016 (Fig. 3). Janampeta is located at extremities of the two ponds which are very big. Mosquitoes find plenty of breeding sources because of the availability of the water source. The village has good irrigation facility; only *Anopheles* and *Culex* reported from this place, with *Cx. gelidus* most dominant. Veerapur is located on the bank of the river Krishna, well irrigated facility, good water sources which act as breeding source for mosquitoes. A total of 1787 mosquitoes were collected. Identification is done by following identification keys of Christopher and Barraud. Three genera and 9 species have been reported so far in these sites. Human dwellings and cattleshed are the most preferred. *An. subpictus*, *Cx. quinquifasciatus*, *Cx. gelidus* are most dominant with 100% distribution. In Mannanur in 2014 *An. vagus*, in 2015 *Ae. albopictus*, in 2016 *An. subpictus* are the dominant species. In

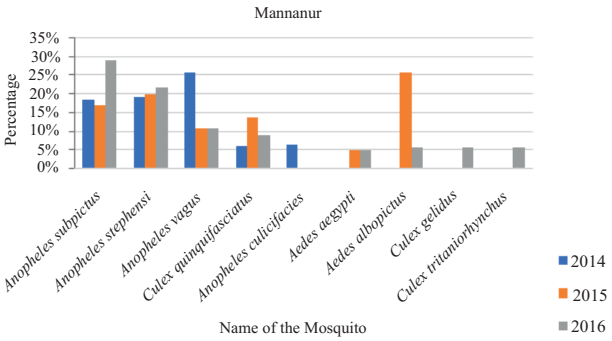

Fig. 3. Density of mosquitoes

Janampeta in 2014 *Cx. gelidus*, are dominant species. In B. Veerapur in 2014 *Cx. quinquifasciatus*, in 2015 and 2016 *Ae. albopictus* are dominant (Fig. 4, 5).

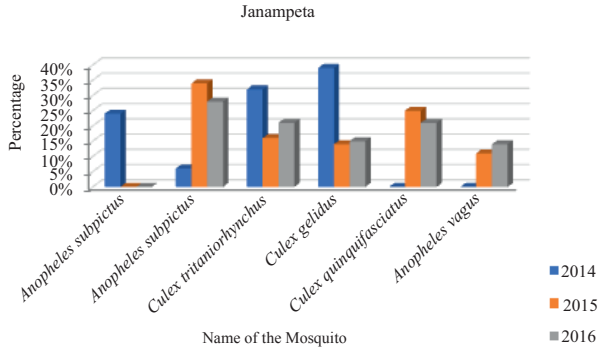

Fig. 4. Janampeta

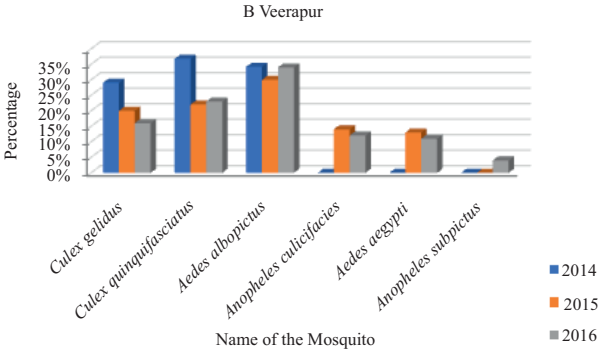

Fig. 5. Veerapur

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EFFICACY OF SEED TREATMENT AND FOLIAR APPLICATION OF AGAINST SOYBEAN STEM FLY *MELANAGROMYZA SOJAE*

C Manjanaik, B Doddabasappa[1*] and M Mallika[2]

Department of Agricultural Entomology, University of Agricultural Sciences,
GKVK, Bengaluru 560065, Karnataka, India
[1]Regional Horticulture Research and Extension Centre, UHS Campus,
GKVK, Bengaluru 560065, Karnataka, India
[2]Department of Extension, University of Agricultural Sciences,
Raichur 584104, Karnataka, India
*Email: dbasappa7096@gmail.com (corresponding author)

ABSTRACT

A field experiment on the effectiveness of seed treatment and foliar application of insecticides against soybean stem fly *Melanagromyza sojae* (Zehntner) was carried out at the All India Coordinated Research Project (AICRP) on Soybean, Gandhi Krishi Vignan Kendra (GKVK), Bengaluru, Karnataka during kharif 2017. The seeds treated with thiamethoxam 30FS @ 10 ml/kg seed and foliar application of thiamethoxam 25WG 0.40 g/ l at 30 days after germination (DAG) were found effective. It was on par with seeds treated with imidacloprid 48FS @ 1.25 ml/ kg seed and foliar application of imidacloprid 17.8SL @ 0.50 ml/ l at 30 DAG. The plots treated with these gave higher grain yield of 1794.48 and 1678.89 kg/ ha, respectively. Higher net gain of 1: 4.22 was obtained from the seeds treated with thiamethaxom 30FS @ 10 ml/ kg seed and foliar application of thiamethoxam 25WG 0.40 g/ l at 30 DAG; and it proved to be highly cost effective. The foliar application of chlorantraniliprole 18.5SC @ 0.30 ml/ l at 10 and 30 DAG was equally effective compared to thiamethoxam and recorded the C: B ratio of 1: 3.94. The plots treated with quinalphos 25EC @ 2 ml/ l at 10 and 30 DAG led to the least stem tunneling of (26.73%) and the low grain yield of 1177.78 kg/ ha.

Key words: Soybean, *Melanagromyza sojae*, thiamethoxam 30FS, thiamethoxam 25WG, seed treatment, foliar application, stem tunneling, yield, C: B ratio

Soybean [*Glycine max* (L.) Merrill] provides 40% protein and 20% edible oil, besides minerals and vitamins, and it has many uses (Roopa and Kambrekar, 2019). India is the fifth largest producer of soybean followed by China. Major soybean producing states are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Uttar Pradesh, Andhra Pradesh and Gujarat. In Karnataka, soybean occupies an area of 0.27 million ha with the production of 0.17 mt and productivity is 639 kg/ ha (Anonymous, 2017). The insect pests often pose a serious threat to the soybean production, by decreasing the yield losses and impairing the quality of the produce (Singh et al., 2000). More than 65 insect species have been reported from Karnataka, infesting the soybean from cotyledon to harvesting stage of the crop (Rai et al., 1973; Adimani, 1976; Thippaiah, 1997). In India, the Stem fly, *Melanagromyza sojae* (Zehn.) (Diptera: Agromyzidae) is emerged as a major insect pest in the soybean at different growth stages (Kundu and Srivastava, 1991; Kumar et al., 2009; Manjanaik et al., 2013; Gaur et al., 2015). The soybean crop is prone to *M. sojae* infestation at all the stages of the crop. The *M. sojae* maggot enters the stem through the leaf petiole and feeds on the stem pith (Van et al., 1998). Crop grown in the sandy soils and under prolonged dry spell prone for severe *M. sojae* infestation and cause 100% crop loss (Talekar and Chen, 1983). The infestation by *M. sojae* on the early stages of the crop growth cause high seedling mortality, and affects the yield (Gangrade and Kogan 1980; Talekar, 1990), and yield losses of 20 to 30% have been reported (Singh and Singh, 1992; Venkatesan and Kundu, 1994; Jayappa, 2000). Foliar application of insecticides is not effective against stem fly, as the larva concealed inside the stem and feed on the internal content of the stem, plant lose the strength and fell on ground. Its presence is detected when plants project the visible symptoms like leaf wither and death of the plant (Kavitha, 2006). The application of insecticides is popular among the farming community due to quick and affective control. Hence, the study was taken on effectiveness of seed treatment and foliar application of insecticides against *M. sojae*.

MATERIALS AND METHODS

The field experiment was conducted at the All India Coordinated Research Project on Soybean, Zonal Agricultural Research Station, Gandhi Krishi Vignan Kendra, Bengaluru (latitude 12°58' N and longitude 77° 35' E, altitude 930 m AMSL). Experiment was laid out in a randomized Complete block design with three replications and eight treatments. The plot size was 3.0 x 3.0 m, the spacing between the rows was 30 cm and between the plants was 10 cm. The soybean cultivar JS-335 was sown in the second week of August during kharif 2017 and followed standard agronomic practices except plant protection measures. The treatments viz., T_1: Seed treatment with thiamethoxam 30 FS @ 10 ml/kg seed and foliar application of thiamethoxam 25WG @ 0.40 g/ l at 30 days after germination (DAG), T_2: Seed treatment with imidacloprid 48FS @ 1.25 ml/ kg seed and foliar application of imidacloprid 17.8SL @ 0.50 ml/ l at 30 DAG, T_3: Seed treatment with chlorpyriphos 20EC @ 5 ml/ kg seed and foliar application chlorpyriphos 20EC @ 2 ml/ l at 30 DAG, T_4: Foliar application of quinalphos 25EC @ 2 ml/ l at 10 and 30 DAG, T_5: Foliar application of lambda cyhalothrin 2.5EC @ 1ml/ l at 10 and 30 DAG, T_6: Foliar application of fipronil 5SC @ 1.50 ml/ l at 10 and 30 DAG, T_7: Foliar application of chlorantraniliprole 18.5SC @ 0.30 ml/ l at 10 and 30 DAG, T_8: Untreated control.

The seeds were treated with the thiamethoxam @ 10 ml/ kg seeds, imidacloprid @1.25 ml/kg seeds and chlorpyriphos @ 5ml/kg seeds. The required quantity of seeds were spread in plastic bowls, prescribed quantity of the insecticide emulsion was then sprinkled on the seeds, the seeds were turned repeatedly to ensure the uniform seed coating with the insecticide. The treated seeds were dried under shade for 30 minutes before sowing. The foliar application of the treatments were taken up with thiamethoxam 25WG @ 0.40 g/ l, imidacloprid 17.8SL @ 0.50 ml/ l and chlorpyriphos 20EC @ 2 ml/ l at 30 days after germination. The insecticides quinalphos @ 2ml/ l, lambda cyhalothrin @ 1 ml/ l, fipronil @ 1.50 ml/ l and chlorantraniliprole @ 0.30 ml/ l were given as foliar application at 10 and 30 days after germination in sequence**.** Observations on the seedling mortality due to stem fly infestation was recorded at 20 and 30 DAG by counting the total number of plants in four rows and the number of plants infested and the infested plants expressed in % seedling mortality. Observations on the stem tunneling were recorded from the ten randomly selected plants. The stem of the plants was split opened vertically with the help of knife. Length of the stem and per cent stem tunneled were recorded. Observations were recorded at 30 days after germination, at maturity and prior to harvesting of the crop. Length of the stem and tunnel length were measured for calculating the % stem tunneling. Harvesting was done at physiological maturity of the crop. The seeds were dried under sunlight for two days to reduce the moisture % and then yield/ plot was recorded and converted into yield in kg/ ha. Prevailing market prices of the produce, cost of the insecticides and cost of laborers were considered for calculating the C: B ratio.

RESULTS AND DISCUSSION

The seeds treated with thiamethoxam 30 FS @ 10ml/ kg seed and foliar application of thiamethoxam 25WG @ 0.40 g/ l at 30 DAG followed by the seeds treated with imidacloprid 48FS @ 1.25 ml/ kg seed and foliar application of imidacloprid 17.8SL @ 0.50 ml/ l at 30 DAG recorded the least seedling mortality of 11.39 and 13.65%, respectively. Both the treatments were equally effective. Foliar application of chlorantraniliprole 18.5SC @ 0.30 ml/ l at 10 and 30 DAG registered 15.18% of seedling mortality. Fipronil 5SC @ 1.50 ml/ l and quinalphos 25EC @ 2 ml/ l were less effective in reducing the seedling mortality. The least plant damage was recorded in the seeds treated with the thiamethoxam 30FS @ 10 ml/ kg seed and foliar application of thiamethoxam 25WG @ 0.40g/ l and it was significantly superior than foliar application of fipronil 5SC @ 1.50 ml/ l. Seeds treated with chlorpyriphos 20EC @ 5 ml/ kg seed and foliar application of chlorpyriphos 20EC @ 2 ml/ l had recorded 19.08% of seedling mortality, which was significantly higher than thiamethoxam 30FS @ 10 ml/ kg seed and foliar application of thiamethoxam 25WG @ 0.40 g/ l. Foliar application of chlorantraniliprole 18.5SC @ 0.30 ml/ l was effective and recorded 15.18% of seedling damage (Table 1).

Significantly low seedling mortality was registered in the seeds treated with thiamethoxam 30FS @ 10 ml/ kg seed and foliar application of thiamethoxam 25WG @ 0.40 g/ l at 30 DAG, it was on par with the seeds treated with imidacloprid 48FS @ 1.25 ml/ kg seed and foliar application of imidacloprid 17.8 SL @ 0.50 ml/ l at 30 DAG. Foliar application of chlorantraniliprole 18.5 SC @ 0.30 ml/ l at 10 and 30 DAG recorded high seedling mortality and it was statistically significant over other treatments. The effectiveness of these insecticides used as seed treatment and foliar application

Table 1. Effectiveness of insecticides against *M. sojae*

Treatment	Seedling mortality (%)	Stem tunneling (%)	Yield (kg/ ha)	C : B Ratio
Seed treatment with thiamethaxom 30FS @ 10.00 ml/kg seed - foliar application of thiamethoxam 25WG @ 0.40g/l at 30 DAG	3.91 (11.39)	6.27 (14.38)	1794.48[a]	1:4.22
Seed treatment with imidacloprid 48FS @ 1.25 ml/kg seed - foliar application of imidacloprid 17.8SL @ 0.50 ml/ l at 30 DAG	5.58 (13.65)	7.65 (15.94)	1678.89[ab]	1:4.19
Seed treatment with chlorpyriphos 20EC @ 5.00 ml/kg seed - foliar application of chlorpyriphos 20EC @ 2ml/ l at 30 DAG	10.70 (19.08)	17.19 (24.46)	1281.48[de]	1:3.24
Foliar application of quinalphos 25EC @ 2.00 ml/ l at 10 and 30 DAG	13.58 (21.62)	20.33 (26.73)	1177.78[e]	1:2.97
Foliar application of lambda cyhalothrin 2.5EC @ 1.00 ml/ l at 10 and 30 DAG	10.88 (19.25)	12.83 (20.92)	1540.73[bc]	1:3.88
Foliar application of fipronil 5SC @ 1.50 ml/ l at 10 and 30 DAG	12.84 (20.98)	15.60 (22.23)	1418.51[cd]	1:3.54
Foliar application of chlorantraniliprole 18.5SC @ 0.30 ml/ l at 10 and 30 DAG	6.87 (15.18)	8.71 (18.34)	1729.62[a]	1:3.94
Untreated control	21.98 (27.96)	32.45 (34.73)	866.67 [f]	-
CD (p=0.05)	2.10	1.67	157.47	-
CV (%)	10.82	7.73	7.42	-

Values given in parentheses arc sine transformation; Means followed by same alphabet statistically on par

observed during the studies are in concurrence with the reports of Gopali et al. (2007), Prabhu and Patil (2016) and Shreedhara et al. (2017). Seeds treated with thiamethaxom 30FS @ 10 ml/kg seed and foliar application of thiamethoxam 25WG @ 0.40 g/ l led to significantly less stem tunneling of (14.38%). Maximum damage of 26.73% was recorded in foliar application of quinalphos 25EC @ 2 ml/ l at 10 and 30 DAG. Whereas, the seeds treated with imidacloprid 48FS @ 1.25 ml/kg seed and foliar application of imidacloprid 17.8SL @ 0.50 ml/ l registered 15.94% stem tunneling. The foliar application of the new molecule chlorantraniliprole 18.5 SC @ 0.30 ml/ l at 10 and 30 DAG registered less stem tunneling of 18.34% which was at par with the lambda cyhalothrin 2.5EC @ 1 ml/ l foliar application at 10 and 30 DAG and it recorded 20.92% of stem tunneling (Table 1).

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PARASITES OF *LEUCINODES ORBONALIS* GUENEE FROM MANIPUR

Sushmita Thokchom*, Ankita Gupta[1], Romila Akoijam[2], and Anjumoni Devee

Department of Entomology, Assam Agricultural University, Jorhat 785013, Assam, India
[1]ICAR-National Bureau of Agricultural Insect Resources, H A Farm Post,
Bellary Road, Hebbal, Bengaluru 560024, Karnataka, India
[2]ICAR Research Complex for North Eastern Hill Region, Manipur Centre,
Lamphelpat 795004, Manipur, India
*Email: pipithockchom@gmail.com (corresponding author)

ABSTRACT

***Leucinodes orbonalis* Guenée (Lepidoptera: Crambidae) is a serious pest of brinjal. During the field observations, a solitary larval parasitoid of *L. orbonalis* viz., *Trathala flavoorbitalis* (Cameron) (Hymenoptera: Ichneumonidae) was osberved. The present study reports larval parasitism of *L. orbonalis* with 6% field parasitism by *T. flavoorbitalis* in Manipur.**

Key words: *Leucinodes orbonalis*, *Trathala flavoorbitalis*, brinjal, larval parasitism, first observation, Manipur

Leucinodes orbonalis Guenée (Lepidoptera: Crambidae) is one of the most notorious pests of brinjal in South and South East Asia; well recorded from India, Bangladesh, Malaysia, Philippines and Sri Lanka (Srinivasan, 2008). For the ecofriendly management use of biological control agents are promising. The genus *Trathala* Cameron has 101 species recorded worldwide (Choi et al., 2014). *Trathala flavoorbitalis*, with type locality from India (Deesa) was originally described as *Tarytia flavo-orbitalis* (Cameron, 1907). This is a well-known parasitoid of *L. orbonalis* and has been documented from Bihar, Tamil Nadu and Karnataka, respectively (Malik et al., 1988; Yasodha and Natarajan, 2006; Murali et al., 2017; Ranjith et al., 2020). This parasitoid species is well distributed globally and is known from the Afrotropical, Australasian, Eastern Palaearctic, Nearctic, Oceanic, and Oriental regions; quite widespread through Indo-Pacific and Eastern Oriental region (Rousse and Villemant, 2012). This parasitic wasp was introduced without establishment into USA and Canada for biological control.

MATERIALS AND METHODS

During 2020-21, surveys were undertaken in the Haorokchambi Leirak, Imphal district of Manipur (24º47'26.3"N,93º55'22.2"E) for checking the infestation and natural parasitism of *L. orbonalis*. During the field observations, a solitary larval parasitoid of *L. orbonalis* viz., *Trathala flavoorbitalis* (Cameron) (Hymenoptera: Ichneumonidae) was noticed. The emerged adult wasps were preserved in 70% alcohol for taxonomic identification. The parasitoids were collected in the laboratory by rearing field collected larvae of *L. orbonalis* in Manipur during December, 2020, with brinjal fruits as diet (28.0°C, 78%RH). The voucher specimens were sent to the National Insect Museum of ICAR-National Bureau of Agricultural Insect Resources (NBAIR) for identification. The morphological studies were carried out in Leica stereozoom SZM S9i microscope.

RESULTS AND DISCUSSION

Trathala flavoorbitalis is known to have numerous hosts belonging to Lepidoptera (Gelechoidea, Noctuoidea, Pyraloidea, Tineoidea and Tortricoidea) (Rousse and Villemant, 2012) and has been reported as a noticeable parasitoid of *Maruca vitrata* (F.) from India (Gupta et al., 2013). The parasitism of *T.flavoorbitalis* in the Indian eggplant fields varies from 3.57-9.06% in Bihar (Malik et al. 1988) up to 40% in Karnataka (Ranjith et al., 2020). *Trathala flavoorbitalis* has a variable colour pattern, mostly orange (appears brown in the dried specimens). It can be identified by yellow scutellum; propodeum yellowish brown in posterior half to brown in anterior half; metasoma with first and second tergites black to dark brown in colour remainder orange-yellow; brownish yellow antennal flagellomeres; ocellar region and rear of vertex black, and apex of hind tibia with dark brown band and wings hyaline. The present study reports revealed that the antenna is longer than the head with 4.916 mm long with the wings 4.396 mm long. *Trathala*

spp. are larval parasitoids of many economically important agricultural pests. The present study reports larval parasitism of *L. orbonalis* with 6% natural field parasitism by *T. flavoorbitalis* in Manipur.

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EVALUATION OF ENTOMOPATHOGENIC FUNGI AGAINST *PEREGRINUS MAIDIS* (ASHMEAD)

D Saicharan* and S S Karabhantanal

Department of Agricultural Entomology, University of Agricultural Sciences Dharwad, College of Agriculture, Vijayapur 586101, Karnataka, India
*Email: charan.dharavath@gmail.com (corresponding author)

ABSTRACT

The present study to evaluate entomopathogenic fungi for the management of sorghum shoot bug, *Peregrinus maidis* (Ashmead) revealed that, among the entomopathogens applied in various methods (foliar spray and whorl application along with FYM), Raichur strain (UASR BC VL 1) of *Lecanicillium lecani* (2×10^8CFU) @ 2g/l directed to whorl region excelled in reducing shoot bug population (5.49/plant), % plant damage (58.62%) and superior plant growth parameters over all other EPF (Entomopathogenic fungi) treatments which was on par results with standard check, cypermethrin 25% EC @ 0.50ml/lit directed to whorl region (5.78/plant & 65.46%). Whereas, all the EPF treatments and untreated control showed superiority in conserving the natural enemy population (spiders and coccinellids) but standard check due to its chemical properties led to the reduction in the beneficial insects with lowest spider (0.54/plant) and coccinellid (0.87/plant) population.

Key words: Coccinellids, Delphacidae, entomopathogenic fungi, farm yard manure, foliar spray, Hemiptera, *Lecanicillium lecani*, *Metarhizium anisopliae*, shoot bug, sorghum, spiders

Sorghum [*Sorghum bicolour* (L.) Moench] is one of the major sources of food for millions of people in tropics and semi-arid tropics of world (Dolly et al., 2019). In India, sorghum is cultivated in an area of 4.80 million ha with an annual production of 4.40 mt (Anon, 2020a) of grain with a productivity of 1005 kg/ha (Anon, 2020b). It has been reported that nearly 150 pests are known to attack the crop at various stages from the day of sowing till harvest (Reddy and Davies, 1979 and Jotwani et al., 1980). Shoot bug *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) is a major sucking pest of sorghum in Northern dry zone of Karnataka. It has been reported that sorghum shoot bug could cause overall loss of, 31.85 % in grain and 33.53 % in fodder yield under unprotected condition during rabi season (Akshatha et al., 2020). In view of these, there is an urgent need to find alternative measures that is friendly to the environment. Among the various components of biocontrol, entomopathogens in general and mycoinsecticides (entomogenous fungi) are most versatile biological control agents. Moreover, there were minimal studies in relation to the management of shoot bug through entomopathogens unlike brown plant hopper. Therefore, efforts were made with the following objectives for the management of *P. maidis* by using entomopathogenic fungi (EPF) in rabi sorghum.

MATERIALS AND METHODS

The studies on the evaluation on entomopathogenic fungi in the management of *P. maidis* was conducted during rabi season using M 35-1 sorghum variety. A field experiment was laid out in Complete Randomized Block Design (CRBD) with three replications during rabi season, 2020-21 at Regional Agricultural Research Station (RARS), Vijayapur, Karnataka, India with following eleven treatments after 30 days after emergence. All the cultural and other operations except plant protection measures were carried out in the experimental plot as per the recommended package of practices (Anon, 2018). SPAD stands for Soil Plant Analysis Development meter which is an equipment which was used to calculate the relative chlorophyll content from the sorghum leaves. SPAD-502 was used as suggested by Markwell et al. (1995). % reduction of shoot bug population over control was worked out using Henderson and Tilton (1955) formula:

$$\text{Corrected \% (1)} = \frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}} \times 100$$

Where, n = Insect population, T = treated, and Co = control

RESULTS AND DISCUSSION

Results indicated that the mean effect of treatments on *P. maidis* incidence and % reduction over control was significantly highest in foliar application of Raichur strain (UASR BC VL 1) of *Lecanicillium lecani* (2x 10^8CFU) @ 2g/ l directed to whorl region (4.44/ plant and 63.49%) and foliar application of commercial *L. lecani* (2x10^8CFU) @ 2g/ l directed to whorl region (5.49/plant and 58.62%) and they excelled their effect over standard check insecticide, cypermethrin 25% EC @ 0.50ml/l directed to whorl region (5.78/ plant and 65.46 (Table 1). These findings were similar to those of Harichandra and Shekharappa (2009) that *M. anisopliae* and *Verticillium lecanii* recorded minimum leafhoppers/ three leaves and maximum yield of 38.80 and 38.50 q/ ha, respectively. Patil et al. (2012) on the efficacy of *V. lecanii* (1.150/0 WP) against sucking pest complex on transgenic *Bt* cotton indicated that Verticel @ 7.50 kg/ ha registered least number of thrips, aphids and leafhoppers and found to be on par with acetamiprid 20SP @ 100 g/ ha. Reddy et al. (2013) stated that *B. bassiana*, *M. anisopliae*, *L. lecani* @ 5 g/ l having 1x10^8 CFU along with standard check- acephate 75% SP @ 1.5 g/ l were effective against *Nilaparvata lugens* Stal in paddy. Against *N. lugens* Chinniah et al. (2016) and Bailal et al. (2020) observed similar results.

Whorl application of Raichur strain (UASR BC Ma 2) of *M. anisopliae* (2x 10^8CFU) @ 1000g mixed with FYM @ 500 kg/ ha (1.64 spiders/plant) and foliar application of commercial *L. lecani* @ 2x10^8CFU (2g/ l-1.41 coccinellids/ plant) is effective and safe to natural enemies. Cypermethrin 25EC @ 0.50 ml/ l was highly toxic to spider and coccinellids and significantly reduced these. These findings are in conformity with reports of Chi et al. (2005) who indicated that predatory spiders and water bugs were higher in fungal treatments. Venkatreddy et al. (2013) reported that *L. lecani* treated plot recorded 9.5 spiders/ hill whereas, chemical treated (acephate 75 SP) plot showed less spider counts of 4.3/ hill and relatively less toxic to predators. Patil et al. (2012) mentioned that, the lower numbers of coccinellids were recorded in standard check (acetamiprid). Results pertaining to plant growth and yield parameters had shown that Raichur strain (UASR BC VL 1) of *L. lecani* (2x10^8CFU) @ 2g/ l directed to whorl region had shown superiority in higher no. of leaves (9.33/ plant), single leaf area (351.71 cm^2), plant height (205.97 cm), relative chlorophyll content (56 SPAD), panicle emergence (92.03%), panicle length (26.10cm), panicle weight (77.32 g), grain yield (2265 kg/ ha) and fodder yield (46.03 q/ ha) which was on par to standard check cypermethrin 25EC @ 0.50 ml/ l. The studies clearly concluded that Raichur strain (UASR BC VL 1) of *L. lecani* (2x 10^8CFU) @ 2g/ l directed to whorl region had shown superiority over all other EPF treatments and on par to the standard check cypermethrin 25EC @ 0.50 ml/ l, which lead to the reduction in shoot bug population, but in contrast to standard check, EPF applied treatments encouraged natural enemies.

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AUTHOR CONTRIBUTION STATEMENT

Dharavath designed, conducted experiments and wrote manuscript. Karabhantanal analysed data. All authors read and approved the manuscript.

CONFLICT OF INTEREST

Authors clearly state that there is no conflict of interest.

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Table 1. Evaluation of entomopathogenic fungi against *P. maidis* in rabi sorghum

Treatments	No. of shoot bugs/ plant*	% reduction of shoot bugs/ plant over control**	No. of natural enemies		% plant damage due to shoot bug at 90 DAS*	No of Leaves per plant	Single leaf area (cm)	Plant height (cm) at harvest	Relative chlorophyl index (SPAD)	Panicle emergence (%) *	Panicle length (Cm)	Panicle Weight (gms)	Grain yield (kg/ ha)	Fodder yield (q/ ha)
			No. of spiders/ plant	No. of cocci-nellids/ plant										
T_1: Whorl application of Raichur strain (UASR BC Ma 2), *Metarhizium anisopliae* (2×10^8CFU) mixed with FYM (1000g + 500 kg/ha)	13.24 (3.71)^{bc}	30.25 (33.37)^{bc}	1.64 (1.46)^{b}	1.23 (1.32)^{a}	16.87 (24.25)^{b}	8.67^{bcd}	338.17^{cd}	186.33^{d}	54.20^{bc}	85.93 (67.97)^{bc}	22.40^{bc}	71.25^{c}	1875^{d}	39.17^{cde}
T_2: Foliar application of Raichur strain (UASR BC Ma 2), *Metarhizium anisopliae* @ 2×10^8CFU (2g/ l) directed to whorl region	11.18 (3.42)^{bc}	29.39 (32.83)^{c}	1.49 (1.41)^{b}	1.34 (1.35)^{a}	14.63 (22.49)^{b}	8.93^{bcd}	341.28^{bcd}	195.4^{bcd}	54.50^{ab}	87.62 (69.40)	23.60^{abc}	72.31^{bc}	1956^{d}	41.45^{bcd}
T_3: Foliar application of Raichur strain (UASR BC VL 1), *Lecanicillium lecani* @ 2×10^8CFU (2g/ l) directed to whorl region	4.44 (2.22)^{a}	63.49 (52.83)^{a}	1.49 (1.41)^{b}	1.23 (1.32)^{a}	10.06 (18.49)^{a}	9.33^{ab}	351.71^{abc}	205.97^{ab}	56.00^{ab}	92.08 (73.65)^{ab}	26.10^{a}	77.32^{ab}	2265^{ab}	46.03^{ab}
T_4: Whorl application of Raichur strain (UASR BC VL 1), *Lecanicillium lecani* (2×10^8CFU) mixed with FYM (1000g + 500 kg/ha)	9.56 (3.17)^{bc}	36.24 (37.01)^{b}	1.54 (1.43)^{b}	1.37 (1.37)^{a}	13.22 (21.32)^{b}	9.03^{abc}	347.03^{abc}	199.84^{abc}	55.70^{ab}	90.57 (72.12)^{ab}	24.40^{abc}	74.87^{bc}	2190^{bc}	43.25^{abc}
T_5: Whorl application of commercial *Metarhizium anisopliae* (2×10^8CFU) mixed with FYM (1000g+500 kg/ ha)	14.37 (3.86)^{c}	25.01 (30.01)^{c}	1.58 (1.44)^{b}	1.31 (1.35)^{a}	16.65 (24.08)^{b}	8.57^{cd}	337.00^{cd}	181.77^{de}	53.80^{abc}	88.65 (70.31)^{b}	22.20^{bc}	70.24^{c}	1790^{e}	37.70^{de}
T_6: Foliar application of commercial *Metarhizium anisopliae* @ 2×10^8CFU (2g/lit) directed to whorl region	11.98 (3.53)^{bc}	37.81 (37.95)^{b}	1.48 (1.41)^{b}	1.35 (1.36)^{a}	17.78 (24.94)^{b}	8.67^{bcd}	346.19^{abcd}	190.2^{cd}	54.80^{abc}	85.67 (67.76)^{bc}	22.90^{bc}	71.13^{c}	1860de	39.65^{cde}

(contd.)

T_7: Whorl application of commercial *Lecanicillium lecani* (2×10^8CFU) mixed with FYM (1000g + 500 kg/ ha)	10.91 (3.38)^{bc}	32.66 (34.85)^{b}	1.54 (1.43)^{b}	1.36 (1.36)^{a}	14.63 (22.49)^{b}	8.67^{bcd}	348.05^{abc}	195.3^{bcd}	55.40^{ab}	88.93 (70.57)^{b}	23.80^{abc}	70.14^{c}	2150^{c}	42.80^{bcd}
T_8: Foliar application of commercial *Lecanicillium lecani* @ 2×10^8CFU(2g/l) directed to whorl region	5.49 (2.45)^{a}	58.62 (49.96)^{a}	1.46 (1.40)^{b}	1.41 (1.38)^{a}	11.87 (20.15)^{ab}	9.00^{a}	348.73^{ab}	200.37^{abc}	54.90^{abc}	90.36 (71.91)^{ab}	25.40^{ab}	75.01^{abc}	2250^{ab}	45.35^{ab}
T_9: Neem based insecticide 1500 PPM @ 4 ml/ l	11.54 (3.47)^{bc}	38.96 (38.62)^{b}	1.28 (1.34)^{b}	1.14 (1.28)^{a}	15.07 (22.84)^{b}	8.67^{bcd}	340.74^{bcd}	196.8^{bcd}	54.40^{abc}	87.89 (69.64)^{bc}	23.60^{abc}	73.62^{bc}	1895^{d}	39.81^{bcd}
T_{10}: Cypermethrin 25% EC @ 0.50ml/lit directed to whorl region	5.78 (2.51)^{a}	65.46 (54.00)^{a}	0.54 (1.02)^{a}	0.87 (1.17)^{b}	8.25 (16.69)^{a}	9.67^{a}	355.91^{a}	208.3^{a}	56.70^{a}	95.61 (77.91)^{a}	26.60^{a}	80.14^{a}	2330^{a}	48.30^{a}
T_{11}: Untreated control (water spray)	35.32 (5.98)^{d}	0.00 (0.00)^{d}	1.52 (1.42)^{b}	1.37 (1.37)^{a}	27.67 (31.74)^{c}	8.33^{d}	333.53^{e}	176.45^{e}	52.90^{bc}	80.35 (63.69)^{c}	22.20^{c}	65.12^{d}	1670^{f}	34.55^{e}
SE.m.±	0.17	1.62	0.02	0.03	1.14	0.21	4.12	3.22	0.74	2.03	0.97	1.68	29.09	1.71
CD (p=0.05)	0.52	4.87	0.07	0.10	3.43	0.63	12.36	9.67	2.23	6.08	2.92	5.05	87.27	5.14
CV (%)	14.41	15.66	12.45	14.33	17.03	11.67	16.02	14.27	14.20	16.33	14.30	13.56	14.90	16.32

Bioagents procured from UAS, Raichur. Commercial bioagents procured from Greenlife Biotech Laboratory. DAA- Days After Application *Figures in parentheses square root transformed/ angular transformed values

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SUSCEPTIBILITY OF LIFE STAGES OF *TRIBOLIUM CASTANEUM* (HERBST) TO MICROWAVE RADIATION

S Raju, B Pathrose, M Chellappan, H Bhaskar and K P Sudheer[1]

Department of Agricultural Entomology; [1]Department of Agricultural Engineering,
College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur 680656, Kerala, India
*Email: berin.pathrose@kau.in (corresponding author)

ABSTRACT

Physical methods of stored grain pest management such as microwave radiation can be utilized as a preventive and curative method of pest control. The susceptibility of various lifestages of *Tribolium castaneum* (Herbst) to microwave radiation was studied by irradiating infested wheat flour at 3 cm flour bed thickness. All the life stages of *T. castaneum* were irradiated at five different power levels viz., 136 W, 264 W, 440 W, 616 W and 800 W for 20 s. Eggs were more susceptible to microwave irradiation, whereas the susceptibility of grubs, pupae and adults were comparable. Mortality of all the life stages of *T. castaneum* increased with the increase in microwave irradiation power. Mortality of egg, grubs, pupae and adults were highest at an irradiation dose of 800 W i.e., 100, 96, 80 and 80%, respectively. Standardization of power, depth of flour bed thickness and duration of irradiation will enable the utilization of household microwave oven for the disinfestation of food grains.

Key words: *Tribolium castaneum*, microwave, irradiation, susceptibility, eggs, grubs, pupae, adult, mortality, red flour beetle, power, wheat flour

Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a cosmopolitan stored grain pest causing severe economic loss to cereal-based products, especially wheat flour. The beetle spoils the wheat flour with chemical excretions, body fragments, and dead insects and renders an off odour to the flour (Rees, 2004). Chemical control is the widely adopted practice for managing red flour beetle. Fumigation is an economical and effective management option. However, the residual effect of chemicals, pest resurgence, resistance development in insects, and adverse environmental impact are the undesirable attributes of chemical control (Abdullahi et al., 2019). Resistance development is a major concern in the storage ecosystem due to the continuous selection pressure imposed by insecticides (Boyer et al., 2012). The frequency of phosphine resistance in Kerala populations of red flour beetle recorded was 71.40 to 93.40% at low concentration and 67.79 to 85.39% at high concentration (Sonai Rajan, 2015). Anusree et al. (2019) reported 10.95fold resistance to malathion in *T. castaneum* collected from different Food Corporation of India (FCI) godowns of Kerala. Such studies extend the constant demand for novel, eco-friendly, and economic strategies for storage pest management. Microwave irradiation is an effective, eco-friendly physical management strategy against storage pests. The dielectric heating generated during microwave irradiation kills the insect and affects the reproductive capacity of surviving insects (Vadivambal et al., 2007; Patil et al., 2020). The current study assessed the efficacy of microwave radiation for the management of *T. castaneum* and studied the susceptibility of each life stage of *T. castaneum* to microwave irradiation.

MATERIALS AND METHODS

The study was conducted during 2021 at the Pesticide Residue Testing Laboratory, Department of Agricultural Entomology, and at the Agri-Business Incubator, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur. The culture of *T. castaneum* was maintained in the laboratory on whole wheat flour with 5 per cent brewer's yeast at 30°C and 70 per cent relative humidity (Pathrose et al., 2005). Adults were sieved out after five days and transferred to fresh rearing jars to obtain uniformly aged insects for conducting various experiments. Each developmental stage of *T. castaneum,* i.e., eggs (two-day-old), grubs (20± 2 day old), pupae and adults (17± 2 day old) of *T. castaneum* were released separately into beakers containing wheat flour at 3 cm bed thickness. Several studies support the use of household microwave oven for the management of storage pest (Ahmady et al., 2016). In our study we used a household microwave

oven (2450 MHz, Morphy Richards 20 MS) at five different power levels (136, 264, 440. 616 and 800 W) for 20 sec. These power levels were chosen as the chosen microwave oven model had these five power levels. Five replications were kept for each treatment. The hatchability of eggs was observed after two weeks by sieving through B.S.S 60 sieve. This data was used to calculate the mortality of eggs. Pupation of grubs was observed five days after treatment, based on which grub mortality was calculated. Similarly, pupal mortality was calculated based on adult emergence from irradiated pupae after five days of treatment. The mortality of adults in each treatment was observed at two days after irradiation. The data obtained were analysed in completely randomized design (two factors- stage of insect and power of irradiation) using an R based statistical package GRAPES, after arcsine transformation (Gopinath et al., 2020).

RESULTS AND DISCUSSION

Analysing the effect of microwave irradiation on various stages, eggs of *T. castaneum* were the most vulnerable stage with significantly high mortality of 51.33%, followed by the larva, adult and pupa (Fig. 1). The mortality of larva (43.33%), adult (40.67%) and pupa (38.00%) did not differ statistically among each other. The susceptibility of different life stages of insects to microwave radiation varies with different species of stored grain pests. Watters (1976) reported that eggs of *T. confusum* were more susceptible to microwave heating followed by pupae, adults and larvae. Shayesteh and Barthakur (1996) also concluded that eggs (12-24 h old) of *T. confusum* was highly susceptible to microwave radiation. Vadivambal et al. (2007) reported that the larvae of *T. castaneum* were more susceptible than pupae and adults to microwave radiation, while the pupae were more tolerant to heat than the other developmental stages. But the study by Vadivambal et al. (2008) in barley damaged by *T. castaneum* revealed that eggs are more susceptible and adults are least susceptible to microwave radiation. The studies conducted on *Callosobruchus maculatus* revealed that eggs were the most vulnerable and pupae were the least susceptible stage to irradiation (Shoughy and Elzun, 2014; Tiwari et al., 2021). In the case of lepidopteran storage pest, fig moth *Ephestia cautella* also, eggs were the most susceptible stage followed by female pupae, larvae and male pupae (Khalaf et al., 2015). Azizoglu et al. (2011) stated that proliferation and development of embryonic cells are slower at the egg stage compared to later developmental stages. This made the eggs to be the most vulnerable stage towards radiation treatment.

The second factor, the power of microwave radiation, caused dose-dependent mortality of *T. castaneum* (Fig. 2). As the microwave power increased from 136 to 800 W, mortality increased significantly from 14.00 to 89.00%, and the mortality in control was zero. Furthermore, several studies on disinfestation of stored product with microwave radiation also supports the positive influence of the power of irradiation on mortality of *T. castaneum* (Vadivambal et al., 2007; Vadivambal et al., 2008; Meenatchi et al., 2015). The interaction effect of life stages and the power of microwave radiation is depicted in Table 1. Egg mortality was complete at the highest irradiation dose of 800 W, when exposed for 20 s. Mortality of grub (96%), pupa (80%) and adult (80%) was also higher at the highest microwave power of 800 W, which was statistically comparable. At 800 W irradiation power 96% mortality of grubs was observed. Mortality of pupae and adults at 800 W were comparable with egg, grub and pupal mortality at 616 W and grub mortality at 440 W. Grub and adult mortality at 136 W and grub

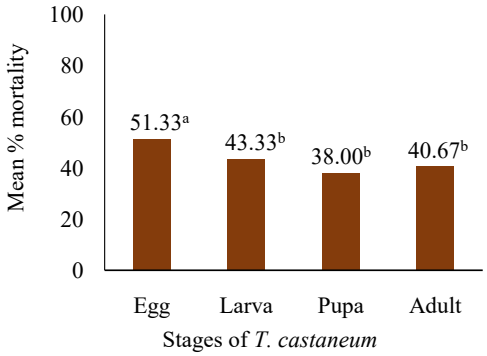

Fig. 1. Susceptibility of life stages of *T. castaneum* to microwave irradiation

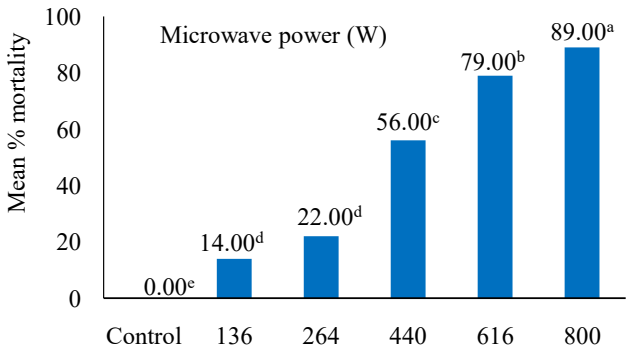

Fig. 2. Effect of microwave power on mortality of *T. castaneum*

Table 1. Effect of life stages/ power of microwave irradiation on mortality of *T. castaneum*

Stage	Power (W)					
	Control	136	264	440	616	800
Egg	0.00^{h}	20.00^{efg}	32.00^{e}	76.00^{cd}	80.00^{bc}	100.00^{a}
	(0.23)	(0.46)	(0.59)	(1.06)	(1.11)	(1.34)
Grub	0.00^{h}	4.00^{gh}	0.00^{h}	80.00^{bc}	80.00^{bc}	96.00^{ab}
	(0.23)	(0.27)	(0.23)	(1.11)	(1.11)	(1.30)
Pupa	0.00^{h}	32.00^{e}	24.00^{ef}	12.00^{fgh}	80.00^{bc}	80.00^{bc}
	(0.23)	(0.60)	(0.50)	(0.37)	(1.11)	(1.11)
Adult	0.00^{h}	0.00^{h}	32.00^{e}	56.00^{d}	76.00^{cd}	80.00^{bc}
	(0.23)	(0.23)	(0.59)	(0.86)	(1.06)	(1.12)

*Figures in parentheses arc sine transformed values; Values with same letters not significantly different (LSD, p=0.05)

mortality at 264 W was comparable to each other and with the control. Vadivambal et al. (2008) reported 100% mortality of *T. castaneum* eggs in barley samples of moisture level 14%, arranged at 1 cm thickness after irradiating at 400 W for 28 s. They recorded cent per cent mortality of grubs, pupae and adults at 500 W, irradiated for 28 s. Higher moisture content leads to a higher temperature level in the irradiated material because of dielectric heating (Qu et al., 2017) and results in higher mortality of insects compared to samples at lower moisture content. Also, microwave irradiation at thin layers results in higher mortality than thick layers (Mohapatra et al., 2014). The low moisture content of 10.66% and a higher bed thickness of 3 cm may have resulted in relatively lower mortality of all stages in the current study compared to the previous studies. The results obtained in the study revealed that microwave irradiation could cause 100% mortality of the eggs of *T. castaneum*. Increasing the duration of irradiation (more than 20s) or reducing the thickness of flour can achieve complete mortality of all life stages of *T. castaneum*.

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NEW RECORD OF A MOSQUITO *AEDES MCINTOSHI* (HUANG) FROM INDIA

Santhosh Goud*, Subasini Pattnaik, Ipsita Biswal, Jaya Kishor Seth, Rupenangshu Kumar Hazra[1], Barsa Baisalini Panda[1], Subbiah Poopathi[2] and Annadurai Bharathi[3]

Post Graduate Department of Zoology, Berhampur University, Berhampur 760007, Odisha, India
[1]ICMR-Regional Medical Research Centre, Bhubaneswar 751023, Odisha, India
[2]ICMR-Vector Control Research Centre, Puducherry 605006, Tamil Nadu, India
[3]PG & Research Department of Zoology, Sir Theagaraya College, Chennai 600021, Tamil Nadu, India
*Email: santhosh1090696@gmail.com (corresponding author)

ABSTRACT

This study reports *Aedes mcintoshi* (Huang, 1985) for the first time from India. It was collected from Berhampur University Campus, District Ganjam, Odisha, India. This species is considered to have medical importance, as it can carry pathogens like viruses and protozoans and can act as a potential vector. Therefore, the present finding of this species will provide baseline information to understand its zoogeography, biology, and pathogenicity related to this mosquito.

Key words: *Aedes mcintoshi*, Culicidae, new record, diversity, zoogeography, vector, medical importance, viruses, protozoans.

Mosquitoes have been a nuisance for humans for over a million years. They are known to spread some of the world's most dangerous diseases like malaria, chikungunya, dengue, filariasis, and Japanese encephalitis. Thousands of people die every year due to these diseases in India (World Health Organization, 2014 and 2020), and nearly one million people die from mosquito-borne diseases worldwide (World Health Organization, 1995a and 1995b). Some of the mosquitoes do not routinely bite humans, but they act as the vectors for several animal diseases and may become dangerous zoonotic agents to spread new diseases. The global mosquito fauna consisted of 3541 species belonging to 50 genera, two subfamilies, and 12 tribes (Tyagi et al., 2015). More than 404 species and subspecies of mosquitoes are available in India, which accounts for more than 12% of the global mosquito fauna (Tyagi et al., 2015). The species *Aedes mcintoshi* is widely distributed in Africa, extending south from sub-Sahelian Africa on both the west and east sides of the continent (WRBU, 2020). The species belongs to the *Lineatopennis* group of the *Aedes* subgenus *Neomelanoconion* together with *A. lineatopennis*, *A. circumluteolus*, *A. luridus*, *A. luteolateralis*, and *A. unidentatus*. For many years, the true identity of *A. mcintoshi* was hidden as this species was misidentified due to its overlapping morphological features with Austro-Oriental species *A. lineatopennis*. Later on, this species was separated from *A. lineatopennis* based on morphological features (Huang, 1985). Landscape genetics approaches supported the single species status of *A. mcintoshi*. However, high genetic variation in subpopulations correlated with soil clay content and recent precipitation (Knight and Hull, 1953).

The species *A. mcintoshi* is a floodwater mosquito, and immatures are typically found in grassy ground pools and residual stream pools. The females bite at night, preferring cattle, but will readily feed on people outdoors. Females deposit their eggs in the upper layer of soil, in grassy sites most likely to flood. Eggs can remain in a dormant state for years, hatching occurs only following sustained submersion (Linley and Turell, 1994). Egg desiccation tolerance in floodwater mosquitoes is often associated with transovarian transmission of viruses between successive generations (Knight and Hull, 1953). The species *A. mcintoshi* is a major vector of Rift Valley fever virus in Africa and also carries other viruses and protozoans like Wesselsbron virus (WSLV), Pongola virus (PGAV), Middelburg virus (MIDV), Ngari virus (NRIV), Ndumu virus (NDUV), Bunyamwera virus (BUNV), Babanki virus (BBKV), and Plasmodium spp. (WRBU, 2020). Laboratory observations suggest that *A. mcintoshi* are effective vector of Rift valley fever virus (Mwaengo et al, 2012). Therefore, it is considered a main communal health concern. However, the study of this mosquito is crucial as it can carry pathogens, including viruses and protozoans. The present finding of the mosquito from Berhampur University campus, Ganjam, Odisha, is the first material evidence of this mosquito from India. The

current record will help us to understand the biology and ecology of this mosquito in future.

MATERIALS AND METHODS

Mosquitoes were collected from the campus area of Berhampur University, Odisha, India (19.2977358°N84.8781602°E). The collection was carried out from January 2018 to December 2019 using an insect battery-operated mechanical aspirator (Pooter) and torchlight. The collected mosquitoes were transferred to a test tube, covered with a loose cotton plug, and examined in the laboratory for identification. Identification of these mosquitoes were made with the help of a 10x fabric lens, and simultaneously the photographs were taken with the help of a mobile camera mounted with a 10x macro lens and L.E.D. Identification of the collected mosquitoes were based on adult characters using standard taxonomic keys and catalogues of Mosquitoes Identification key of Christophers (1933), Barraud (1934), and online identification keys from Walter Reed Biosystematics Unit (WRBU) Website: http://wrbu.si.edu/vectorspecies/mosquitoes/mcintoshi. The identity of the mosquito was confirmed with Indian Council of Medical Research-Regional Medical Research Centre, Bhubaneshwar and Indian Council of Medical Research-Vector Control Research Centre, Puducherry. These mosquitoes were deposited and registered in the national repository of Estuarine Biology Regional Centre, Zoological Survey of India, Gopalpur-on-Sea, Odisha, India.

RESULTS AND DISCUSSION

Aedes mcintoshi (Huang, 1985) (Fig. 1)

Redescription

Head narrow decumbent scales on the vertex;

Fig. 1. *Aedes mcintoshi*: A. Habitus, B. Lateral view, C. Dorsal view, D. Bare spiracular area, E. Scutum with yellow scales laterally, F. Head, G. Wing with dark scales and fringed squama, H. Pointed abdomen showing tergum with pale basal bands

numerous erect forked scales on the head, not limited to the occiput. Palps are short, proboscis is long, and the palps and proboscis are both dark. Thorax: From the anterior promontory to the scutellum, the scutum is surrounded by a lateral band of yellow scales; the scutellar scales are all narrow; the acrostichal and dorsocentral setae are present; the paratergite is bare; and the mesepimeron has a lower anterior seta. Wings: Squama fringed. Dark scaled except pale scales on basal radius vein. Legs: Hind femur mostly dark with pale ventrally on basal 2/3. Tibia and tarsi are dark. Abdomen: Whole abdomen pointed. Terga with basal pale bands (Fig. 1A-H)

Materials examined: 17 examples, Registration Number: EBRC/ZSI/In-12259 A-Q, collected by: Santhosh Goud, Adults**:** ♀ Medium sized; body length 0.6 to 0.7 cm.

Distribution: South Africa, Angola, Botswana, Democratic Republic of the Congo, Ethiopia, Gambia, Kenya, Mali, Nigeria, Republic of South Africa, Senegal, South Sudan, Tanzania, Uganda, Zambia, Zimbabwe (WRBU, 2020).

Remarks: The species *A. mcintoshi* was first described by Huang (1985), and bionomics, distribution and larval forms were documented by Knight and Hull (1953) as *A. lineatopennis*. Earlier this species was misidentified as *A. lineatopennis* (Ludlow) from African regions, which is considered to be widespread in Oriental and Afrotropical regions. Later on, in 1985 it was discriminated from *A. lineatopennis* by Huang based on differences in the wing scale pattern and male genitalia. The presence of the mosquito *A. mcintoshi* from Odisha constitutes the first material evidence of this mosquito from the Indian subcontinent. This finding will provide baseline information to understand the zoogeography, biology, and pathogenicity related to this mosquito.

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AUTHOR CONTRIBUTIONS STATEMENT

S Goud conducted the survey, collected the specimen, taken the photograph of the specimen. Identified by S Goud and J K Seth, I Biswal, B B Panda, S Pattnaik, R K Hazra, S Poopathi prepared the manuscript. All authors read and approved the manuscript.

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SEASONAL INCIDENCE OF MAJOR INSECT PESTS OF SESAME

Neeraj Kumar*, M L Sharma and Naveen

Department of Entomology, College of Agriculture,
Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior 474002, Madhya Pradesh, India
*Email: neeraj333yadav@gmail.com (corresponding author)

ABSTRACT

This study explores the seasonal incidence of major insect pests of sesame at the Entomology Research Farm of the College of Agriculture, RVSKVV, Gwalior, Madhya Pradesh during kharif, 2019. The results revealed that- among the sucking pests, the incidence of leafhopper *Orosius albicinctus* exhibited a positive and significant correlation with evening relative humidity (RH) and rainfall; and with evaporation it was significantly negative. The whitefly *Bemisia tabaci* did not show any significant correlation with weather factors. Of the other major pests, incidence of horned caterpillar *Acherontia styx* was observed to be significantly positively correlated with rainfall, evening RH and minimum temperature whereas it was a significant negative one with evaporation. With leafroller and capsule borer *Antigastra catalaunalis* it was a significant positive correlation with morning/ evening RH and rainfall, and a significant negative one with evaporation. Pod damage by *A. catalaunalis* was significantly negatively correlated with maximum and minimum temperature. The mirid *Nesidiocoris tenuis* showed a positive correlation with morning RH, and a significantly negative one with maximum temperature and evaporation. The incidence of gall fly *Asphondylia sesami* revealed a significant positive correlation with morning RH, while it was a significant negative one with evaporation and maximum temperature.

Key words: *Sesamum indicum*, sesamum, seasonal incidence, *Antigastra catalaunalis*, *Orosius albicinctus*, *Bemisia tabaci*, *Nesidiocoris tenuis*, *Acherontia styx*, *Asphondylia sesami*, weather factors, seasonal incidence

Sesame (*Sesamum indicum* L.) is an indigenous oilseed crop of India with Madhya Pradesh, Gujarat, Rajasthan and Uttar Pradesh being the major sesame growing states. In Madhya Pradesh, it has a yield of 293 kg/ ha (Anonymous, 2017), which is seriously affected by both biotic and abiotic factors. Of the biotic factors, the major constraint is the damage by insect pests (Egonyu et al., 2005; Ahirwar et al., 2010), causing 25 to 90% loss in seed yield (Ahuja and Kalyan, 2001). The crop is attacked by 29 insect pests in different stages of its growth (Biswas et al., 2001). Ahuja and Bakhetia (1995) documented 65 insect pests and one species of mite. Among various insect pests viz., leaf roller and capsule borer, *Antigastra catalaunalis* (Duponchel); Jassid, *Orosius albicinctus* (Distant); whitefly, *Bemisia tabaci* (Gennadius), mirid bug, *Nesidiocoris tenuis* (Reuter), til hawk moth, *Acherontia styx* (Westwood); Bihar hairy caterpillar, *Diacrisia obliqua* (Walker); sesame gall fly, *Asphondylia sesami* (Felt) are the key pests of regular occurrence (Sasikumar and Sardana, 1988; Nath et al., 2002; Ahirwar et al., 2009; Biswas and Das, 2011; Thangjam and Vastrad, 2015). Nymphs and adults of *O. albicinctus* and *B. tabaci* suck the plant sap in addition to transmitting phyllody and leaf curl diseases (Ahirwar et al., 2010); *A. catalaunalis* is the most serious pest causing yield losses of up to 90% (Ahuja and Bakhetia, 1995). The concept of IPM requires the basics of pest ecology, and area or region-specific strategies will need details of seasonal incidence. The present study explores the seasonal incidence of major insect pests of sesame in Madhya Pradesh.

MATERIALS AND METHODS

The study was conducted at the farm of the College of Agriculture, RVSKVV, Gwalior (26°14'N, 78°15'E, 211.52 masl) during kharif 2019. The healthy seeds of sesame variety TKG-506 were sown manually on 27th July 2019 with a spacing of 30 cm between rows and 10 cm between plants. The weather data were sourced from the Department of Agricultural Meteorology, College of Agriculture, Gwalior. The incidence of insect pests was recorded at weekly intervals on ten randomly selected tagged plants starting from germination till harvest. Counts of *B. tabaci* and *O. albicinctus* were made from the upper, middle and lower leaves, while that of *A. styx* moth larvae were just counted/ plant; for *A. sesami*, randomly selected 50 capsules/ plot were observed to count healthy and damaged ones; number of larvae on webbed leaves

was counted for the *A. catalaunalis* with 50 capsules/ plot used for assessing damaged capsules/ 10 plants. Observation on shoot tips infected by *N. tenuis* count was recorded on 10 randomly selected plants. All the data were subjected to ANOVA with critical difference values computed. Correlation of incidence of pests was done with weather factors using Microsoft Excel.

RESULTS AND DISCUSSION

During the study six insect pests were observed viz., *B. tabaci*, *O. albicinctus, A. styx, A. catalaunalis, N. tenuis* and *A. sesame.* The incidence of *B. tabaci* was first observed during the 32nd standard meteorological week (SMW) (2 whiteflies) and it gradually increased and reached its peak during 36th SMW (20) and thereafter started decreasing and reached its lowest level during the 44th SMW (8) (Fig. 1). These observations corroborate with those of Thakur et al. (2019), in Bundelkhand Zone of Madhya Pradesh. Similar findings have been reported by Choudhary et al. (2015), Sharma (2017), Ahirwar et al. (2009) and Mishra et al. (2015). The correlation coefficient of incidence of *B. tabaci* population with weather parameters were non-significant. Choudhary et al. (2015) and Sharma (2017) too observed a non-significant positive correlation with rainfall. Thakur et al. (2019), Patidar (2010) and Sharma (2017) also reported similar relationships. The first incidence of *O. albicinctus* was during the 32nd SMW with (12 jassids), and it reached its maximum during 36th SMW (42), and reached its lowest during the 44th SMW (14). These observations are in agreement with those of Ahirwar et al. (2009), Gangwar et al. (2014), Choudhary et al. (2015), Mishra et al. (2015), and Sharma (2017). The seasonal incidence of *O. albicinctus* in the Bundelkhand Zone of Madhya Pradesh started in 31st SMW with a peak in 38th SMW as shown by Thakur et al. (2019); incidence of this pest was observed to be positively and significantly associated with evening RH (0.45) and rainfall (0.59) while its correlation with evaporation (-0.48) was significantly negative. Gangwar et al. (2014), Mishra et al. (2015) and Sharma (2017) observed a non-significant correlation with maximum temperature; and Patidar (2010) and Choudhary et al. (2015) observed a significant correlation with minimum temperature and RH.

The first occurrence of larva of *A. styx* was during the 33rd SMW with 5 larvae; this increased from 33rd SMW and reached its peak during 36th SMW (16 larvae) and thereafter started decreasing and reached its lowest during 43rd SMW (1 larva) (Fig. 1). Bondre et al. (2016) observed a peak activity of *A. styx* during the 2nd week of October (41st SMW) with a maximum number of larvae observed during the vegetative stage. These corroborate with the observations of Biswas et al. (2001). The correlation analysis of its incidence revealed a significant positive correlation with rainfall (0.62), evening RH (0.56) and minimum temperature (0.53), whereas it was a significant negative one with evaporation (-0.44) (Table 1). Bondre et al. (2016) observed such significant correlation with maximum and minimum temperature, RH, vapour pressure and evaporation. Results of Ahuja and Kalyan (2001) also are in line with the present ones. Incidence of *A. catalaunalis* was first observed during the 32nd SMW with 4 larvae; it increased from 32nd SMW with peak during 37th SMW (21) and it was minimum during 43rd and 44th SMW (5) (Fig. 1); in capsules (8.0%) it was observed during the 36th SMW with a peak during the 41st SMW (53% damage). These results corroborate with those of Thakur et al. (2019). Mishra

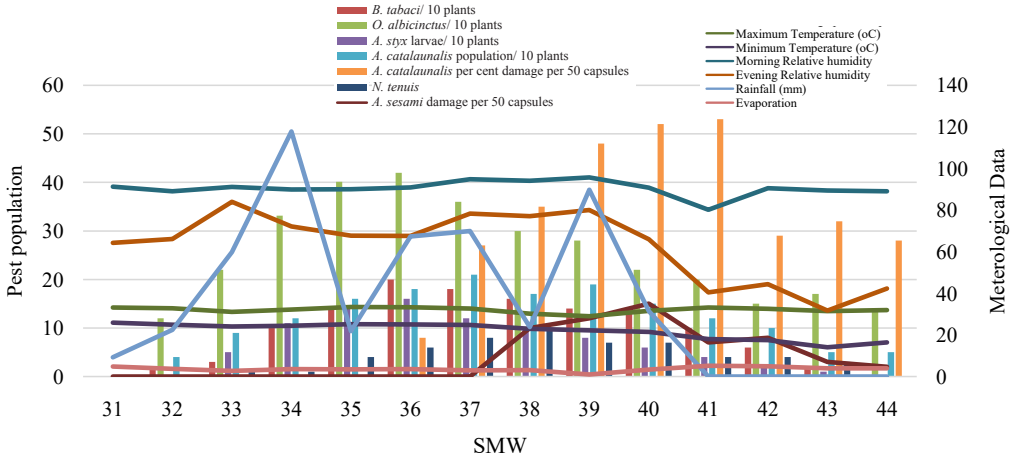

Fig. 1. Seasonal incidence of major insect pests of sesame (kharif, 2019)

Table 1

S. No.	Weather factor	Major insect pests of sesame (kharif, 2019)						
		B. tabaci	*O. albicinctus*	*A. styx*	*A. catalaunalis*		*N. tenuis*	*A. sesami*
					Incidence	Damage		
1	Maximum temperature	-0.09^{NS}	-0.02^{NS}	0.05^{NS}	-0.23^{NS}	-0.45*	-0.37*	-0.37*
2	Minimum temperature	-0.01^{NS}	0.35^{NS}	0.53**	0.28^{NS}	-0.59**	0.07^{NS}	0.07^{NS}
3	Morning RH	0.22^{NS}	0.27^{NS}	0.33^{NS}	0.38*	-0.11^{NS}	0.42*	0.42*
4	Evening RH	0.13^{NS}	0.45*	0.56**	0.49*	-0.32^{NS}	0.31^{NS}	0.31^{NS}
5	Rainfall	0.29^{NS}	0.59**	0.62**	0.53**	-0.22^{NS}	0.18^{NS}	0.18^{NS}
6	Evaporation	-0.32^{NS}	-0.48*	-0.44*	-0.56**	-0.10^{NS}	-0.42^{NS}	-0.42*

*Significant at p=0.05; ** p=0.01; NS- Non significant.

et al. (2015) and Muzaffar et al. (2002) also provide similar conclusions. A significant positive correlation of its incidence was observed with morning (0.38) and evening RH (0.49) and rainfall (0.53); while it was a significant negative correlation with evaporation (-0.56) (Table 1); with capsule damage it was a significant negative correlation with maximum (-0.45) and minimum temperature (0.59). Kumar and Goel (1994) observed such a significant negative correlation of larval counts with minimum temperature. Kumar et al. (2012), Vishnupriya et al. (2003) and Bondre et al. (2016) corroborate these observations. However, Thakur et al. (2019) revealed a significant positive correlation with maximum temperature (0.48) and a significantly negative correlation with RH (-0.71).

The *N. tenuis* first appeared during the 33rd SMW (1 nymph) reached its peak at 38th SMW (10 nymphs) and reached its least at 43rd and 44th SMW (3 and 0) (Fig. 1); in Bundelkhand Zone it started appearing in 31st SMW with a peak in 35/ 36th SMW (Thakur et al., 2019; Mishra et al., 2015). The correlation analysis revealed a positive correlation of its incidence with morning RH (0.42), and a significant negative one with maximum temperature (-0.37) (Table 1), as observed by Mishra et al. (2015),and Thakur et al. (2019). The incidence of *A. sesami* was observed during the 38th SMW (10% capsules damage), which reached its maximum at 40th SMW (15%), and the least during 44th SMW (Fig. 1). These results are in accordance with those of Kumar et al. (2010). Bondre et al. (2016) revealed that the activity of *A. sesame* was during the 2nd week of November (41st SMW). The correlation analysis revealed a significant positive correlation between its damage and morning RH (0.42), and a significant negative one with evaporation (-0.42) and maximum temperature (-0.37) (Table 1). Ahuja and Kalyan (2001), Kumar et al. (2010) and Bondre et al. (2016) support these results.

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PESTICIDE RESIDUE ANLYSIS IN EXCRETA OF SPOTTED OWLET *ATHENE BRAMA* AND BARN OWL *TYTO ALBA*

Yashika Gaba and Nisha Vashishat*

Department of Zoology, Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: nisha.vashishat@pau.edu (corresponding author)

ABSTRACT

The continuous use of pesticides to improve agriculture has not only affected the crop but also altered the food chain and worst affected the non-target organisms. Birds utilizing agricultural landscapes for feeding get exposed to these chemicals through ingestion of sprayed soils, treated granules or seeds and prey items. Raptors like owls are the most likely victims of pesticide exposure as they are at the top level of food chain. In present study, excreta samples of barn owl and spotted owlet were collected from three locations and tested for presence of pesticide residues using gas chromatography. The organophosphate residues of dichlorvos, monocrotophos, phorate, malathion, quinalphos, profenophos, ethion were not reported in excreta of both owl species but the residues of chlorpyriphos (0.037 ppm) were detected in spotted owlet samples collected from village Barnhara only. The pyrethroid residues cyhalothrin, permethrin, cypermethrin were also found to be absent or below detectable limit in samples collected from different locations. The feeding habits of birds attribute to the level of contamination in predatory birds. Although the level of chlorpyriphos residues excreted out from the body of spotted owlet does not reflect the actual level of exposure to this bird but gives an indication of their being at the risk of exposure in the environment.

Key words: Pesticides, excreta, contamination, food chain, predatory bird, environment, residues, feeding habits, agricultural landscapes, exposure, spotted owl, barn owl

Pesticides are the chemicals that are released intentionally into the environment to suppress the pests and protect agricultural and industrial products. Increasing demand of continuous food supply has resulted in excessive use of these chemicals. However, most of these do not specifically target a particular pest but also affect the non-target species. They can have short term harmful effects on the organisms facing direct exposure or long-term effects by entering the food chain. Birds are highly migratory species, so it is impossible to exclude them from areas treated with more pesticides. Avian species dependent on agricultural landscapes for food may get exposed to pesticides through consumption of treated seeds, granules, or sprayed soils and prey items (Eng et al., 2017). Agricultural areas in India probably experience the heaviest and indiscriminate use of chemical pesticides leading to direct and indirect mortality of predatory and frugivorous birds (Dhindsa et al., 1986). A visible decline has been witnessed in abundance of birds as a result of alteration and reduction in their feeding, nesting and breeding grounds (Isaksson, 2018) Agroecosystem of Punjab, for the many past decades, has been subjected to tremendous change due to deforestation, intensive agriculture and excessive use of pesticides along with urbanization and industrial growth which in turn affected the avian community resulting in the reshuffling of many species of birds.

Pesticides become lethal at higher concentrations and result into density-dependent indirect effects (Fleeger et al., 2003). At sub-lethal concentrations, they show alterations in bird's morphology, physiology, hormones, reproduction, neurotransmitters, immune response and behavior including predator detection and swimming ability (Abrams, 1995). These chemicals can disrupt the central nervous systems, alter behaviour, cause endocrine system dysfunctions, affect immune systems, and inhibit growth in living organisms (Mitra et al., 2011). The growing concern has recently focused on the indirect effects of pesticides on aves besides their lethal and sub lethal effects. These effects act mainly by reducing food supplies (weeds, invertebrates), especially during breeding or winter seasons. Insecticides and acaricides primarily affect bird populations by reducing the availability of their arthropod prey. Still, the consumption of contaminated prey, e.g. ants contaminated with DDT, locust contaminated with fenitrothion may cause the deaths of insectivorous birds through acute poisoning or causes

sub-lethal effects which will affect their behaviour or breeding success. Birds and mammals are not able to excrete easily the metabolites of organochlorine pesticides due to their lipophilic nature which results into their accumulation in adipose tissues and biological magnification at the higher trophic level (Mitra and Maitra, 2018). When it comes to birds of higher levels in the food chain, like raptors they develop abnormalities like shell-less eggs or sometimes thinner eggs hells. This, in turn, affects the different birds' reproductive capacity (Vishnudas, 2007). Birds of prey like owls, being at the top of food chain are the most likely victims of pesticide and chemical contamination. Like other predatory birds, owls are adversely affected by indiscriminate use of pesticides in modern agriculture. The population of these owl species therefore seems to be declining in many agricultural areas like Punjab, indicating a collapsing ecosystem.

The assessment of the exposure of birds to toxic compounds needs some non-invasive methods. Moreover, the capturing and killing of birds is legally banned by Govt. of India according to the Wildlife Protection Act 1972; therefore any analytical studies on the tissues and eggs of these organisms are beyond the reach of scientists working in this area. Thus, excreta of bird are an alternative source, which if analyzed can assess the harmful impact of environmental contaminants on these organisms and in our previous studies bird excrements have been used successfully as non-destructive indicators of chemical contamination in birds' diet (Sharma and Vashishat, 2017; Gaba and Vashishat, 2018). Therefore, keeping in view the beneficial role of spotted owlet and barn owl in agricultural areas, the present study was carried out to provide information on the level of their exposure to environmental contaminants through analysis of excreta.

MATERIALS AND METHODS

The collection of dry excreta samples of spotted owlet and barn owl was done from their from the roosting and nesting sites at three locations of district Ludhiana i.e. location I- village Barnhara situated near Buddha Nullah, location II- village Ladhowal situated near river Sutlej and location III- agricultural field areas of Punjab Agricultural University. Acetone, dichloromethane, hexane, sodium sulphate and activated charcoal (LR grade) were redistilled in all glass apparatus. The analytical technical grade pesticide standards i.e. heptachlor, quinalphos, chlorpyriphos and triazophos were used and procured from Sigma-Aldrich (USA). Five gram pooled excreta sample of each species was weighed and dipped in 50 ml acetone overnight. The extracts were filtered with rinsings of acetone in funnel containing 250 ml of 5% sodium chloride solution followed by addition of 75 ml of dichoromethane to collect lower layer. Second rinsing was given with 75 ml of hexane to collect upper non aqueous layer. The different fractions were combined and then treated with 100 mg of activated charcoal (in powder form) for about 2-3 hours at room temperature. The clear extracts so obtained were filtered and concentrated in a rotary evaporator to 15 ml. The extracts were cleaned up by using the column chromatography with the help of silica gel as an adsorbent which was activated at 110º C for 2 hours before use. A glass column was packed with activated silica gel (made of 20g silica +1g charcoal) in between the two small layers of anhydrous sodium sulfate supported on a plug of glass wool. The column was pre-washed with dichloromethane and the extract was poured over it. The extract was eluted with a freshly prepared solvent mixture of dichloromethane-acetone (1:1). The elute was concentrated to near dryness in a rotary evaporator under vacuum and then transferred to 5 ml acetone for further analysis.

The pesticide sample extracts were analyzed using gas chromatograph (GC) equipped with electron capture detector (Agilent 7890B System) and flame thermionic detector (Shimadzu model 2010). For electron capture detector the initial temperature of GC oven was set at 170º C for 13 min followed 20 by increase in temperature to 270º C at the rate of 3º C per minute with hold time for 20 min. The initial oven temperature for flame thermionic detector was set at 150º C with hold time for 5 min followed by increase in temperature to 220º C at the rate of 10º C per minute with hold time for 5 min, and the final temperature was made up to 250º C at the rate of 5º C per minute with hold time for 13 min. The injection port temperature was set at 280º C whereas the temperature of both the detectors was adjusted at 310º C. Nitrogen was used as carrier gas while hydrogen and air were used for flame formation. Calibration curves for all standards of organochlorines (OCs), organophosphates (OPs) and synthetic pyrethroids (SPs) were drawn for concentration versus area of the peak and the correlation coefficients (r2) were determined near to 0.99. The residues were quantified by using the standard formula (Van Coot et al., 2018).

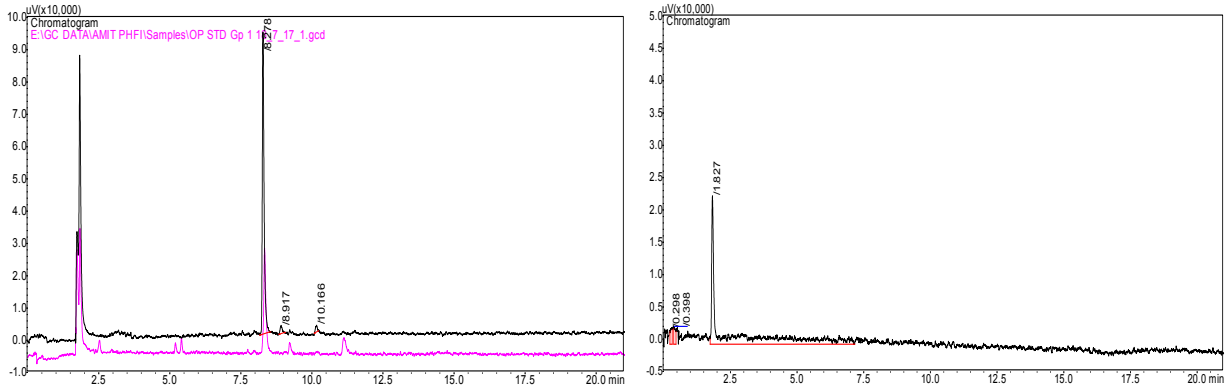
RESULTS AND DISCUSSION

The 5 g of dry excreta samples of spotted owlet and barn owl were collected and pooled from each

site. The samples were then analyzed for detection of pesticide residues like Organophosphates and synthetic pyrethroids. The OP residues of Dichlorvos, Monocrotophos, Phorate, Malathion, Quinalphos, Profenophos, Ethion were found to be absent in excreta of both owl species but the residues of chlorpyriphos were detected in samples of spotted owlet only collected from village Barnhara. Other OP residues were not detected which may be because of low biomagnification properties in comparison to other pesticides like OCs. Jayakumar et al (2020) studied the presence of OCs with highest accumulation of hexachlorocyclohexane in tissues of colonial nesting birds in sanctuary of Tamil Nadu, India. Further, Dhananjayan et al (2020) observed OC residues in abandoned eggs of 22 terrestrial avian species from Tamil Nadu, India. The OPs and carbamates which are commonly used pesticides throughout the world have low bioaccumulating capacity in food chain and less persistence. This may be a reason for non-detection of many of the organophosphates in present study. The OPs, until activated in the liver by microsomal oxidation enzymes do not become potent inhibitors of cholinesterase. They are generally less toxic than other pesticide groups. Birds are particularly sensitive to the toxic effects of organophosphorus and carbamate pesticides (Hill, 1995). Isenring (2010) reported OPs namely chlorpyrifos, diazinon, isofenphos, malathion, mevinphos, phorate and carbamates namely aldicarb, bendiocarb, carbofuran as a cause of fatal bird poisoning. The pyrethroid residues Cyhalothrin, Permethrin, Cypermethrin were also found to be absent in samples collected from different locations. The synthetic pyrethroids are one of the least toxic insecticides. The different routes of exposure to different chemical pollutants can decrease the capacity of avian species to excrete these chemical residues through excreta. The residues of only chlorpyriphos i.e. 0.037 pm were detected in samples of spotted owlet collected from village barnhara (Fig. 1a) however, no such pesticide residue was observed in excreta samples of barn owl (Fig. 1b).

Although the level of chlorpyriphos residues excreted out from the body of spotted owlet does not reflect the actual level of exposure to this bird but gives an indication of their being at the risk of exposure in the environment. Birds in agricultural environments are commonly exposed to the insecticides, mainly through ingestion of invertebrates after insecticide application (Crisol-Martínez et al., 2016). The reason for its presence may be that the chlorpyriphos, because of its insecticidal property, is used for the control of ticks in cattle and buffaloes. It is also sprayed in crop fields for control of insect pests. In a study conducted by Malhotra and Singla (2018) on analysis of regurgitated pellets of spotted owlet from Punjab, India the diet of spotted owlet mainly consisted of insects i.e. 53.8% which indicates the entry of pesticide through food chain. However, the diet of bam owl consisted only of vertebrates, in which 88 per cent were rodents alone (Malhotra and Singla, 2017). The absence of insects in the diet of barn owl may be a reason behind the non-detection of pesticide residues in excreta of this bird.

Chlorpyriphos, an OP, inhibits acetyl choline esterase enzyme in a way that has cross generational implications (Anway et al., 2005) and it severely affects birds (Mitra et al., 2011). In order to monitor the pesticide residue in birds, the metabolic studies are required not only to identify the primary metabolites but also their accumulation and distribution within the body (Katagi and Fujisawa, 2021). Eng et al. (2017) have reported the impairment of migration



(a) (b)

Fig. 1. Chromatogram for pesticide residue analysis of excreta of a) Spotted owlet from village Barnhara; b) Barn owl from village Ladhowal

ability in white-crowned sparrow, a seed eating bird, after exposure to low (10% LD_{50}) and high dose (25% LD_{50}) of chlorpyriphos. The non detection of the other pesticide residues in excreta samples revealed that the residues of OPs and pyrethroids can be absent in faecal samples. The feeding habits of carnivorous birds actually attribute to the level of contamination in predatory birds (Van Drooge et al., 2008; Gupta et al., 2017). In general, birds that eat other birds, or fish, have higher residues than those that eat seeds and vegetation. Birds of prey were primarily affected; exceptions apparently are the result of lesser exposure because of different food habits. The detection of chlorpyriphos in the excreta of Spotted Owlet highlights its potential as bioindicator of pesticide contamination in birds of prey. Furthermore, the present study can serve as baseline for future research in general as well as control values during the analysis of samples obtained from birds in the event of suspected pesticide poisoning.

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AUTHOR'S CONTRIBUTION STATEMENT

NV conceived and designed research. YG conducted the experiments and collected data. NV and YG interpreted the results and wrote the manuscript. Both authors read and approved the manuscript.

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DETERMINATION OF TETRANILIPROLE AND ITS METABOLITE RESIDUES IN TOMATO

J Kousika, K Bhuvaneswari* and V Muralitharan

Department of Agricultural Entomology, Tamil Nadu Agricultural University,
Coimbatore 641003, Tamil Nadu, India
*Email: bhuvanetnau@gmail.com (corresponding author)

ABSTRACT

Dissipation of tetraniliprole and its metabolite, chinazolinon in tomato was studied. Immature fruit samples were collected at specific intervals with three applications. Modified QuEChERS method was used and satisfactory recovery of 78.28 to 104.77 % was obtained. Good linearity (0.05 to 1.00 µg g^{-1}) with coefficient of determination (R^2) > 0.99 was recorded. The initial deposit at 60 (x) and 120 g a.i. ha^{-1} (2x) was 0.42 and 0.65 µg g^{-1} and reached the below limit of quantification on 5th and 7th days after spraying. The half-life period was 1.3 and 1.6 days and the safe waiting period was 4.5 and 5.8 days at x and 2x doses, respectively. The harvest time (20th day) samples, mature fruit and soil were at BLQ in both the doses. The metabolite, chinazolinon was not detected in any of the sample analyzed. Theoretical maximum residue contribution (TMRC) was less than maximum permissible intake even on the day of spraying.

Key words: Tomato, soil, fruit, tetraniliprole, metabolite- chinazolinon, dissipation, half -life, safe waiting period, hazard index, modified QuEChERS method, linearity, coefficient of determination

Tomato is a major tropical and subtropical vegetable and in India, the area under cultivation and the annual production is about 781 thousand ha and 4976 thousand mt, respectively (NABARD, 2019). Tomato is susceptible to many insect pests and diseases and the major pest include fruit borer *Helicoverpa armigera* (Hubner), aphids *Aphis gossypii* (Glover), whitefly *Bemisia tabaci* (Gennadius), leaf eating caterpillar *Spodoptera litura* (F.), thrips, *Thrips tabaci* Lindeman, American serpentine leaf miner *Liriomyza trifolii* (Burgess) and two spotted red spider mite *Tetranychus urticae* Koch (Misra, 2010). Of these, the fruit borer is the most damaging, and numerous insecticides are employed for its control. One such insecticide with novel mode of action is tetraniliprole which showed an increased efficacy against lepidopteran caterpillars, dipterans and was found to be relatively safe to non-target organisms (Ramesh et al., 2016; Mahla et al., 2017). Tetraniliprole is an insecticide belonging to the phthalic acid diamide group developed by Bayer Crop Science (Bayer Annual Report, 2016). This compound binds and activates the ryanodine receptors and reduces intracellular calcium reserves, resulting in muscle paralysis and death (CIBRC, 2021). Repeated application of such insecticides in the later stage of fruit development may result in insecticide residues in the harvested fruits. This study aimed to determine the persistence of tetraniliprole and its metabolite chinazolinon residues in tomatoes under the Tamil Nadu Agroclimatic conditions and to estimate the dietary risk assessment of tetraniliprole.

MATERIALS AND METHODS

The formulation tetraniliprole 200SC, its certified reference standard (99.70% purity), and its metabolite chinazolinon (99.70% purity) (M/s. Bayer Crop Science, Mumbai), solvents viz., acetonitrile (Lichrosolv and Chromosolv grade), methanol (Chromosolv grade), salts viz., anhydrous NaCl and analytical grade sodium sulfate (M/s Merck Bangalore, India), anhydrous magnesium sulphate ($MgSO_4$) (M/s. Himedia Laboratory, Mumbai), primary secondary amine (PSA, 40 µm, Bondesil), graphitized carbon black (GCB) (M/s. Agilent, USA), LCMS grade formic acid (M/s. Sigma Aldrich., Bangalore) and membrane filter paper (0.45 and 0.20 µm, M/s. Pal life Science, Mumbai) were purchased. The ultra-pure type - I (18.2 MΩ) water was prepared in the laboratory using Merck (Direct - Q® 3) water purifier. A supervised field experiment was conducted at Muttathuvayal, Coimbatore (10.9624°N, 76.7445°E) to investigate the dissipation pattern of tetraniliprole in tomato from January to May 2017. Tetraniliprole 200 SC was applied at 60 (x) and 120 g a.i. ha^{-1} (2x) using power- operated knapsack sprayer thrice at ten days interval starting from fruit initiation stage. Each treatment was replicated thrice by following randomized block design (RBD) with a plot size of 25 m^2, with a separate plot for untreated control. After the

third application, immature fruits were collected at 0 (within 2 hr of the last application), 1, 3, 5, 7, 10 and 15th day. During harvest time (20 DAT), mature fruit and soil samples were collected. One kg of immature tomato fruits was collected randomly from treated and untreated plots. Soil samples were collected from randomly chosen spots after clearing surface litters and samples were drawn at 0 to 15 cm depth using a conical trier. The foreign material (roots, stones, gravels and pebbles) in collected soil were removed and a subsample of 250 g was shade dried. The collected fruit and soil samples were labeled, packed and brought to the laboratory.

Insecticide stock solution was prepared in a 25 ml class A volumetric flask. Tetraniliprole and its metabolite, chinazolinon were weighted accurately to 10.03 mg within a volumetric flask and using acetonitrile, volume was made up to the mark to get 400 µg g^{-1} and stored in -20^{0}C for further use. Six repeated injections of tetraniliprole and chinazolinon at concentrations ranging from 0.05 to 1.0 µg g^{-1} were used to determine the linearity. The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-background noise ratio. To determine the accuracy of the method, tetraniliprole and its metabolite, chinazolinon residues was fortified at 0.05, 0.25 and 0.5 µg g^{-1} with three replications in a 10 g tomato fruit and soil and processed for further analysis as mentioned below. Recovery (%) was computed and the precision was defined as % relative standard deviation (RSD). The fruit samples collected for residue analysis were chopped and blended in a high-volume blade homogenizer, Robot CoupeÒ. The residues of tetraniliprole and its metabolite were processed using the modified QuEChERS (dispersive solid phase extraction) method (Meenambigai et al., 2017).

Tetraniliprole and its metabolite chinazolinon were examined for residues using the liquid chromatography mass spectrometry (Shimadzu- LCMS-2020) system equipped with electrospray ionization (ESI) fitted with a reverse phase Shimadzu shim-pack GIST-HP C18 column, 100 x 3.0 mm with 3 µ particle size. Tetraniliprole and its metabolite chinazolinon were quantified using mass spectra of 543 m/z (-ESI) and 528 m/z (+ESI), respectively. A low-pressure gradient programme was set with the mobile phase ratio of A: degassed water with 0.1% formic acid (60%) + B: degassed methanol 0.1% formic acid (40%) and A: degassed water with 0.1% formic acid (70%) + B: degassed methanol 0.1% formic acid (30%) with the flow rate of 0.6 and 0.8 ml min^{-1} for tetraniliprole and its metabolite chinazolinon, respectively. The column temperature was 40^{0}C and the injection volume 20 µl. The MS parameters were; interface temperature 350^{o}C; DL temperature 250^{o}C; heat block temperature 200^{o}C; nebulizing gas flow was 1.5 l/ min. and dry gas flow 15 l/ min. The retention time was 8.7 and 11.4 min for tetraniliprole and its metabolite, respectively showing clear separation of parent and its metabolite by the method. The risk assessment associated with the consumption of tetraniliprole treated tomato was determined by comparing theoretical maximum residue contribution (TMRC) with maximum permissible intake (MPI) (FAO, 2009). The daily consumption of tomatoes for Indian men and women (200 g) as well as the average human body weight of an Indian men (60 kg) and women (55 kg) were referred from NIN (2011). The ADI of tetraniliprole is 0.06 mg kg^{-1} body weight day^{-1} (Anonymous, 2019). The residue data was statistically analyzed to calculate the half-life (Hoskins, 1961). The safe waiting period was determined using the formula mentioned by Handa et al. (1999); safe waiting period = [log (A)-log (LOQ)]/K

RESULTS AND DISCUSSION

A simple and easy method was validated for extracting tetraniliprole and chinazolinon residues from tomato fruit and soil. Good recovery (70 to 120%) and RSD (20%) were obtained, satisfying the SANTE, 2017 guidelines. Calibration curve was obtained by injecting five different levels of both tetraniliprole and the metabolite chinazolinon (0.05, 0.1, 0.25, 0.5 and 1.0 µg g^{-1}) with six replications (Fig. 1). Both tetraniliprole and its metabolite chinazolinon exhibited good linearity (R^2 > 0.99). LOD (signal to noise ratio of 3) and LOQ (signal to noise ratio of 10) of tetraniliprole and its metabolite chinazolinon in tomato fruit and soil were determined to be 0.01 and 0.05 µg g^{-1}, respectively. The recovery percentage of tetraniliprole and chinazolinon in tomato fruit and soil ranged from 78.28 to 104.77 with the RSD of 2.91 to 15.32% (Table 1). Kaushik et al. (2019) reported the recovery as 94.00 to 120.80 for tetraniliprole and 92.80 to 119.60% for chinazolinon in tomato and soil, respectively using non QuEChERS method for cleanup. The calculated residues of tetraniliprole and chinazolinon are depicted in Table 2. The initial tetraniliprole deposits on tomato fruits were 0.42 and 0.65 µg g^{-1} at X and 2X doses, respectively. On the third day after spray, more than 50% of the initial deposit dissipated and reached Below Limit of Quantification (BLQ) (0.05 µg g^{-1}) at 5th and 7th days after treatment in recommended and double doses, respectively. A graph was computed based on the sampling intervals and log

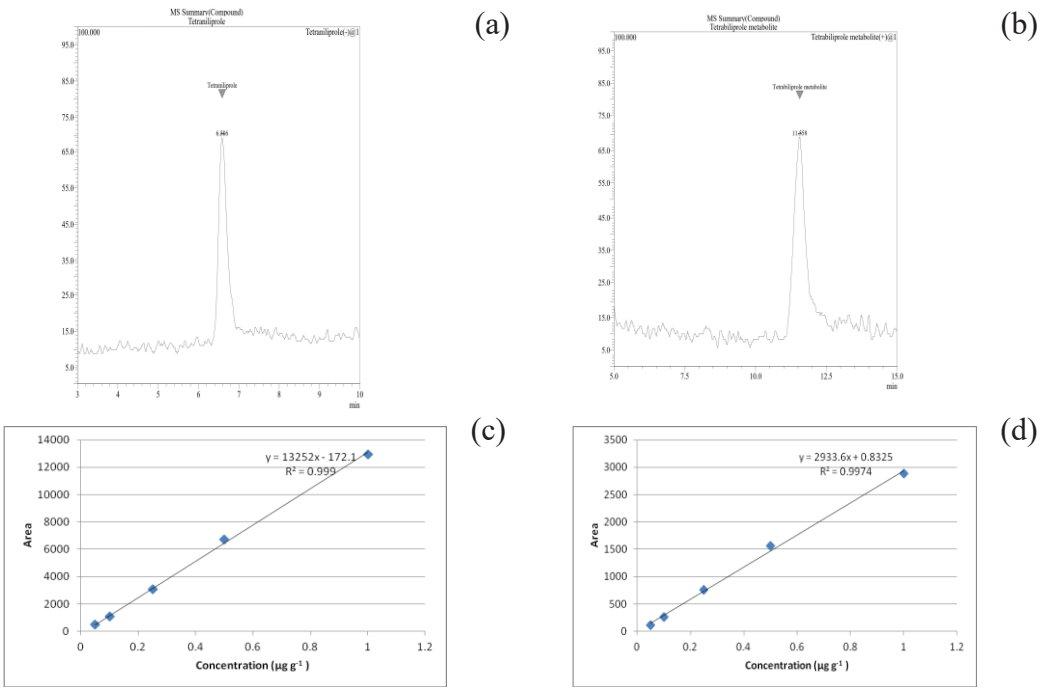

Fig. 1. The Standard chromatogram of tetraniliprole (a) & chinazolinon (b) at LOQ level and linearity chromatogram of tetraniliprole (c) & chinazolinon (d)

Table 1. Recovery of tetraniliprole and its metabolite in tomato fruit and soil

Fortification (µg g^{-1})	Tetraniliprole		Chinazolinon	
	Fruit	Soil	Fruit	Soil
	*Mean % recovery (RSD %)	*Mean % recovery (RSD %)	*Mean % recovery (RSD %)	*Mean % recovery (RSD %)
0.05	101.19 (2.91)	102.16 (6.93)	87.06 (15.32)	94.81 (6.43)
0.25	86.39 (5.17)	104.77 (5.02)	94.63 (8.48)	90.58 (2.91)
0.50	78.28 (5.96)	96.37 (7.86)	93.29 (6.90)	85.06 (6.40)

*Mean of three replications

Table 2. Dissipation kinetics and dietary risk assessment of tetraniliprole in tomato

Days after spraying	Tetraniliprole 200SC 60 g a.i. ha^{-1} (X dose)			Tetraniliprole 200SC 120 g a.i. ha^{-1} (2X dose)			MPI (mg/ person/ day)	
	Mean residue* (µg g^{-1})	Dissipation %	TMRC (µg/ person)	Mean residue* (µg g^{-1})	Dissipation %	TMRC (µg/ person)	Adult male (65kg)	Adult female (55kg)
0	0.42	-	0.08	0.65	-	0.13	3.90	3.30
1	0.30	29.15	0.06	0.43	34.43	0.09		
3	0.09	78.42	0.02	0.26	59.38	0.05		
5	BLQ	-	-	0.07	89.68	0.01		
7	BLQ	-	-	BLQ	-	-		
Mature fruit	BLQ	-	-	BLQ	-	-		
Soil	BLQ	-	-	BLQ	-	-		
R^2	0.987	-	-	-	0.926	-		
$t_{½}$ (days)	1.31	-	-	-	1.64	-		
Safe waiting period	4.48	5.75	-			-		
Regression equation	Y= 2.692-0.268x	-	-	Y= 2.874-0.219x	-	-		

*Mean of three replications, BLQ- below limit of quantification

values of average residue, to estimate the regression. The regression equation estimated the half – life period as 1.21 (X dose) and 1.64 days (2X dose). This shows the faster degradation behavior of the insecticide even at a higher dose. Since no MRL is available for tetraniliprole, safe waiting period calculation was done using LOQ and arrived as 4.48 days for single dose and 5.75 days for double doses. The harvest time samples *viz*., mature fruit and soil collected at 20 days after treatment were found to be BLQ (< 0.05 µg g^{-1}). The residue of the metabolite, Chinazolinon was not detected in any of the tomato fruit or soil samples collected and analyzed.

In a field study conducted at Solan, the half life period of tetraniliprole in tomato was reported as 2.70 and 3.49 days with the safe waiting period of 11.77 and 14.86 days (Kaushik et al., 2019). Plant character (type, form, growth, plant surface, crop stage, kind of fruit surface and texture) and environmental factors (humidity, temperature and rain) influences the initial deposit and persistence of pesticide (Ebling, 1963). In the present study, the mean maximum temperature recorded during the experimental period was 36.2^{0}C which might have favored the faster degradation of tetraniliprole residue. Volatilization is one factor favoring degradation and influenced by higher temperature plays a vital role in dissipation of pesticide from crops which shares 90% of the total loss of applied dose (Tepper, 2017). The safety of tetraniliprole residues was studied by comparing the TMRC values with the MPI. The TMRC value of tetraniliprole, on the day of spraying (within 2 hrs) was determined as 0.01 µg $person^{-1}$ at recommended and 0.02 µg $person^{-1}$ at double the recommended dose. TMRC values were far less than the MPI value for both adult male and female (3.90 and 3.30 mg/ person/ day) (Table 2).

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EFFICACY OF GAMMA RADIATION ON THE GREATER WAX MOTH *GALLERIA MELLONELLA* L.

Mohsin R Khan*, A Vijay and Barkat Hussain[1]

Department of Physics, GLA University, Mathura 281406, Uttar Pradesh, India
[1]Division of Entomology, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology-Kashmir, Shalimar 190025, Srinagar, Kashmir, India
*Email: shaanmohsin1@gmail.com (corresponding author) ORCID ID: 0000-0001-6820-1413

ABSTRACT

The effect of ionizing radiation from 50 to 450 Gy on the eggs and larvae 100 to 1500 Gy. on larvae of greater wax moth *Galleria mellonella* L. The results revealed that the egg hatchability reduced to 50% at 102.70 Gy. In the most radiation resistant eggs, 350 Gy resulted in no pupal formation while a dose of 250 Gy resulted in no adult formation. Within 51.53 hr, a dose of 500 Gy reduced the larval survival to 50%. With 148, 574 and 680 Gy, the proportion of larvae that matured into adults reduced to 50%, 10%, and 0%, respectively. A radiation dose between 300 and 400 Gy was found adequate for egg sterilization and larval mortality.

Key words: *Galleria mellonella, g*amma radiation, lethal dose, radiation dose, 50-1500 Gy, egg hatchability, pupation, adult formation, egg sterlization, larval mortality

The greater wax moth (*Galleria mellonella* L.) is a well-known pest that harms honey bee colonies. It causes significant economic losses of 60-70% to Indian beekeepers (Hanumanth et al*.,* 2009). Roughly US$ 4.5 million dollars is lost in United States, with about US$ 1 million occurring in Florida alone (Kondrateva et al*.,* 2020). In tropical and subtropical areas, *G. mellonella* is the limiting factor that severely harm honey bee populations (Charles et al., 2017), it occurs due to various reasons, viz; poor nutrition, illness, the loss of the queen, or widespread pesticide poisoning (Pirk et al., 2015). *Galleria mellonella* also contributes to the spread of infectious illnesses, including the foulbrood (Goulson et al., 2015). Management of this pest has drawn significant interest (Shimanuki et al., 1980; Williams, 1997). *Galleria mellonella* has historically been subjected to heat and cold treatments to end all stages of its existence (Bombelli, 2017; Charles et al., 2017). Chemical pesticides used detrimental side have effects on non-target organisms and preradiation is used to combat this problem (Hallman and Blackburn, 2016). A dose of 400 Gy phytosanitary irradiation is necessary to destroy wax moth eggs (Mansour, 2020). However, significant research on the dose required to kill the larvae has not been quantified.

In the current study, an effort was made to apply gamma radiation to combat *G. mellonella* with a view to generate baseline data calculating for LD_{50}) values for the eggs and larvae. This study looked at several variables, including how gamma radiation affects egg hatching, pupation, and adult emergence. Additionally, the effects of gamma radiation on the irradiated larvae that develop into pupae and adult stages were evaluated, as well as the fluctuation in mortality over time for the larvae exposed to various dosage levels.

MATERIALS AND METHODS

The Department of Nematology at CCSU in Meerut, Uttar Pradesh, provided the *G. mellonella* culture. The culture was maintained in a growth chamber with controlled humidity and temperature. The culture was raised on an artificial diet (Firacative et al., 2020). Eggs and larvae of *G. mellonella* were collected by the standard procedure (Mansour, 2020). The age of the eggs was noted from the first day till egg hatch. Different age groups' of eggs and larvae received varying levels of radiation. A gamma irradiation chamber (model Gamma Cell Elite-I) with a Cs-137 radioactive source was used. Paper cups containing eggs attached to paper strips were put into the irradiation chamber, while larvae were directly introduced into the paper cups. The eggs were returned to the BOD incubator and reared on diet medium. Within two weeks, some of these eggs hatched and became larvae. After five weeks, some larvae began to weave cocoons around themselves to develop into pupae. To produce adults, the pupae were housed in sterile containers. Adult food in the form of a 10% sucrose solution was provided. Radiation doses used ranging from 50 to 450 Gy with an incremental dose of 50 Gy were administered to eggs of various age

groups (1-6 days old). For the following 10 days, the irradiated eggs were watched to count hatching eggs. A mixed population of larvae was separately exposed to radiation at doses ranging from 100 to 1500 Gy with a 100 Gy increment. To help with the computation of the LD_{50} dose, which kills 50% of the exposed larvae within 24 hr, the irradiated larvae were observed for the mortality. Four samples of 50 each, were used for each dose. A mixed population of larval instars was selected to reflect the real scenario of an infected comb. To determine the LD_{50} dose with the larvae subjected to doses of 500, 1000 and 1500 Gy were observed for the following 96 hr. The % mortality was documented every 24 hr. Data on the mortality and the sterility of irradiated eggs were subjected to probit analysis using IBM's SPSS software. The same software was used to perform Pearson's chi-square test to determine the significance of the relationship between dosage and mortality. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine the data's normality.

RESULTS AND DISCUSSION

It was observed that the *G. mellonella* eggs are more radiosensitive while they are young and eventually become resistant as they age, but, as the exposure increased, the resistance reduced (Fig. 1). For eggs that were irradiated at 1-2, 3-4, and 5-6 days of age, respectively, the regression results demonstrated a substantial inverse relationship between gamma radiation dose and egg hatch (P 0.05, $R^2 = 0.916, 0.877$, and 0.954). The most radio-resistant eggs were discovered to be 5-6 days old, whereas the radio-sensitive eggs were found to be 1-2 days old. A dose of 250 Gy resulted in 0% egg hatch in the 1-2 days old eggs and reduced the egg hatch to 12.75 and 29.50%, respectively in 3-4 and 5-6 days old eggs. The egg hatch reduced significantly with dose (Table 1). A dose of 50Gy affected the survival of larvae ($p < 0.05$) in most radio-resistant eggs (5-6 days old eggs)

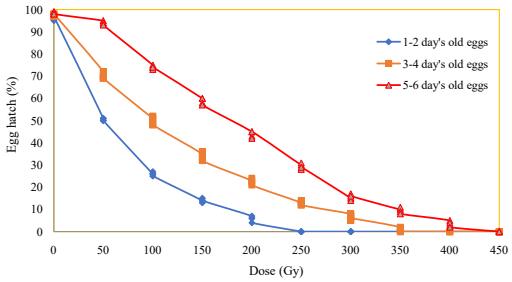

Fig. 1. Effect of ionizing radiation on % egg hatch of *Galleria mellonella*

and a dose of 350Gy completely arrested the survival of larvae. For the age groups of 1-2, 3-4, and 5-6-day-old eggs, respectively, a strong negative connection between the dose and survival to adult stage was found ($p < 0.05$, $R^2 = 0.979, 0.988, 0.906$). For a mixed population of the larval instars, it was observed that a dose of 350Gy reduced the irradiated larvae becoming pupae to 50%. Doses of 148, 574 and 680Gy reduced the irradiated larvae turning into adults to 50, 10 and 0%, respectively ($p < 0.05$, $R^2 = 0.698$) (Fig. 2). The graph clearly shows a relationship between the proportion of mortality over time and radiation exposure. The mortality of larvae exposed to doses of 500, 1000 and 1500 Gy was 21.72, 41.63 and 63.98%, respectively, at 24 hr. A dose of 500 Gy caused 83% larval mortality after 96 hr and lowered the survival to 50% after 51.53 hr.

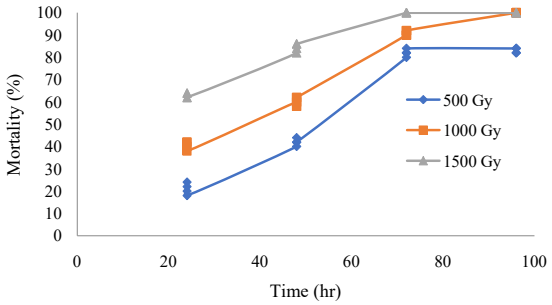

Fig. 2. Effect of ionizing radiation on cumulative larval mortality in *Galleria mellonella*

Table 1. Variation of survival with dose on the age of the irradiated eggs of *Galleria mellonella*

Dose (Gy)	1-2 days old eggs			3-4 days old eggs			5-6 days old eggs		
	Survival to larval stage	Survival to pupal stage	Survival to adult stage	Survival to larval stage	Survival to pupal stage	Survival to adult stage	Survival to larval stage	Survival to pupal stage	Survival to adult stage
50	50.50%	41.25%	39.00%	70.00%	60.25%	56.50%	93.75%	80.50%	73.75%
100	26 .00%	24.50%	22.75%	50.00%	42.50%	39.25%	74.00%	60.50%	53.75%
150	14.00%	15.75%	8.75%	34.00%	28.25%	25.00%	58.75%	36.75%	29.75%
200	5.75 %	8.75%	0.00%	22.50%	11.50%	8.75%	43.75%	23.75%	16.75%
250	0.00%	0.00%	0.00%	12.75%	5.00%	0.00%	29.50%	13.50%	8.50%
300	0.00%	0.00%	0.00%	6.50%	0.00%	0.00%	15.50%	3.25%	1.50%
350	0.00%	0.00%	0.00%	0.75%	0.00%	0.00%	9.50%	0.00%	0.00%
400	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	3.50%	0.00%	0.00%
450	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Use of ionizing radiation for pest disinfestations started in the early 20th century (Runner, 1916). Phytosanitary irradiation of various insect pests with a dose ranging from 150 to 400 Gy is being used extensively (Follett et al., 2022). The present results indicate a dependence on the dose. These findings are similar to those of Nadel et al. (2018), Jafari et al. (2010) reported that the most effective dose for the sterilization of the male pupae of *G. mellonella* as 350 Gy. The present results are in close agreement with those of Hallman et al. (2010); Mansour (2020); Ayvare in contrast to those of Milcheva az et al. (2008); Mansour and Al-Attar (2012) on many lepidopteran pests. The observation on doses for different parameters are close to that of Mansour (2010; 2015; 2016). The LD_{50} observed now are in contrast to those of Milcheva (2004) and it might be due to various reasons (Hallman, 2000; White et al., 1977). Essentially, ionizing gamma radiation has quarantine potential for the management of the *G. mellonella* and a dose of 300 to 400 Gy is quite satisfactory.

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POPULATION DYNAMICS OF INSECT PESTS ASSOCIATED WITH CABBAGE AND CAULIFLOWER AND FARMERS' FRIENDLY IPM

Bharat Singh, Neetu Singh* and Satyendra Singh[1]

Amity Institute of Organic Farming; [2]Amity Centre for Bio Control and Plant Disease Management, Amity University Uttar Pradesh (AUUP), Sec-125, Noida 301303 Uttar Pradesh, India
[1]ICAR-National Centre for Integrated Pest Management, IARI, Pusa, New Delhi 110012, India
*Email: nsingh19@amity.edu (corresponding author): ORCID ID 0000-0001-6094-7421

ABSTRACT

A field survey in the farmers' fields at Gurugram in Haryana, in few blocks focused on the incidence of pests of cabbage and cauliflower during rabi, 2017-18 and 2018-19. These revealed the occurrence of *Plutella xylostella* and *Spodoptera litura.* Maximum number of larvae of *P. xylostella* and *S. litura* was recorded in Pataudi block as compared to those of Farukhnagar, Sohana and Gurugram. Seven neem-based biopesticides were evaluated along with check carbosulfan at hot spots. Data revealed that NSKE 5% was quite effective followed by Nimbicidin and Neemgold. The cost benefit ratio was maximum with NSKE (1:9.41, 1:9.53 for cabbage and cauliflower, respectively) followed by neem leaf extract (NLE) with (1:7.72 and 1:6.31). These results conclude that NSKE and NLE can be recommended against major pests of cabbage and cauliflower.

Key words: Cabbage, cauliflower, *Plutella xylostella, Spodoptera litura*, incidence, biopesticides, Nimbicidin, Neemgold, NSKE, neem leaf extract, yield, cost benefit ratio

Vegetables constitute an important part of our daily human diet. Due to high content of protein and carbohydrates, these crops are prone to attack by insect pests, and crop loss is estimated to be around 52-100% (Anuradha, 1997; Cardleron and Hare, 1986). Talekar (1992) states that the annual cost of managing these pests globally is estimated to be one billion US $. Cabbage and cauliflower are infested by a number of insect pests which can cause substantial yield losses. Among the lepidopteran pests, diamond back moth, *Plutella xylostella* and tobacco caterpillar, *Spodoptera litura* are the most important causing direct reduction in yield and indirectly in quality. Cabbage aphids *Brevicoryne brassicae* and cabbage white butterfly *Pieris brassicae* cause leaf damage up to 31% (Mochiah et al., 2011). A single factor cannot be considered to estimate the population pattern of *P. xystella* and *S. litura* on cabbage and cauliflower in Haryana. Emphasis is being given to alternative method of controlling these. Efforts are being made to bring in effective, ecofriendly IPM measures acceptable to farmers. Application of botanicals and biopesticides are effectively used against *P. xylostella* (Srinivasan and Krishna, 1991; Saravaiya, and Patel, 2005). The present study explores the insect pests associated with cauliflower and cabbage and few ecofriendly IPM measures using neem formulations in Gurugram, Haryana.

MATERIALS AND METHODS

A roving survey of Dist. Gurugram, Haryana was carried out in the infested area covering four different blocks viz. Pataudi (Uncha Majra, Bhora Kalan, Narhera, Khanpur, Mamtajpur, Baspadmka villages) Farukhnagar (Fazilpur Tajnagar, Farukhanagar, Kaliavas villages) Sohna (Baluda, Garhimurli, Sohna ki Dhani, Badshahpur villages) and Gurugram (Chandu, Garhi Harsaru, Garhi Gopalpur, Sardana villages) during rabi, 2017-18 and 2018-19. Besides, collection of insects, the farmers of each block were interviewed, and their views were noted. These identified areas were visited every week, and insects collected. Under field conditions, three seedlings of cabbage and cauliflower were kept covered with glass chimney and open end was tied with muslin cloth for aeration, and replicated thrice. After 40 days of growth, one two and four insect larvae of tobacco caterpillar, *S. litura* and diamond back moth, *P. xylostella* were put in each chimney having healthy growth of plants for observing damage symptoms in cauliflower and cabbage. A field trial was conducted against *P. xylostella* and *S. litura* in cauliflower and cabbage with eight treatments with three replications in a plots of size of 10x 10 m in block Pataudi during both the years. The objective was to evaluate the efficacy of neem formulations. Seedlings were prepared in nursery of cauliflower cv. Pusa snowball K1 and cabbage cv.

Golden Acre and seedlings were transplanted after 30 days, with sprays given by knapsack sprayer, first 30 days after transplantation and second after 15 days of first spray. Observations were recorded on the incidence of pests, and efficacy of treatments was estimated based on % yield increase over control. The treatments evaluated include: Neemazal, Neem Gold, Neemol, Nimbicidine, neem leaf extract (NLE), NSKE, Rakshak @ 5 ml/ l each and carbosulfan 2 ml/ l, along with control (without treatment). The yield data were recorded at harvest time and further subjected to cost benefit ratio.

RESULTS AND DISCUSSION

The results revealed that *P. xylostella* and *S. litura* were the major pests causing damage up to 70-80%. These were monitored in cabbage and cauliflower in four blocks i.e. Patandi, Farukhnagar, Sohna and Gurugram (Haryana) during rabi 2017-18 and 2018-19; the pooled mean number of larvae of *P. xylostella* and *S. litura* showed significant differences among four blocks; *P. xylostella* larvae on cauliflower and cabbage was 10.9 and 7.20/10 plants, respectively in Pataudi block, being maximum (Table 1). Similar trend in number of larvae of *S. litura* was observed in Pataudi (5.03 and 4.88 larvae/ 10 plants), Farukhnagar(4.25 and 3.30 larvae/ 10 plants), Sohana (3.60 and 4.13 larvae/ 10 plants) and Gurugram (2.38 and 2.78 larvae/ 10 plants). Thus, Pataudi block showed maximum incidence (Table 1). Chimney controlled plant growth technique resulted minute observations on symptoms and damage by *P. xylostella* and *S. litura*; *P. xylostella* larva is small and green, feed on leaves makes bite holes, and causes excessive defoliation on primordial, causing yield loss up to 70-80%; *S. litura* eggs are laid in clusters on under surface of leaves, and young larvae feed gregariously and skeletonize the leaves, while large larvae bore into heads, causing yield loss of 60-70%. *P. xylostella*) is an important pest and causes extensive damage to the cabbage and cauliflower (Devi et al., 1995); *S. litura* damaged seedlings, disrupts head formation. The presence of larvae of *P. xylostella* and *S. litura* causes in rejection of marketable produce (Kranz et al., 1977).

The acceptance of any of any botanical based IPM module in cauliflower and cabbage depends upon its economics; the product cost, labour charges and expenditure of application of each spray varied from Rs 2462 to Rs 6150/ ha, due to difference in cost of biopesticide, botanical preparations, and insecticides. Data given in Table 2 based on pooled analysis, reveal that NLE was at minimum cost Rs. 2462/ ha and net profit of Rs. 35261/ ha and Rs. 34018/ ha were obtained in cauliflower and cabbage, respectively with two sprays of Nimbicidine. This is followed by NSKE with net profit of Rs. 29435, 29076/ ha, respectively in cauliflower and cabbage crops; Neemazal gave minimum net profit of Rs. 14995/ ha and Rs 14627/ ha in cauliflower and cabbage, respectively. The maximum incremental cost benefit ratio of 1:9.53 and 1: 9.41 was obtained with NSKE, while in others it varied from 1: 2.98 to 1: 6.84 and 1: 2.91 to 1: 7.72, respectively in cauliflower and cabbage. Singh (2006) evaluated antifeedant process of Nimbicidine against *S. litura* larvae at high concentration and it showed more deterrency with least feeding area on leaves. Pal et al. (2015) confirmed that against *Bactrocera cucurbitae,* NSKE @ 5 ml/ l showed maximum efficacy. Nazrussalam et al. (2008) observed that NLE @ 5 ml/ l showed maximum deterrence and the highest yield of cauliflower and cabbage.

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Table 1. Incidence of *P. xystella* and *S. litura* on cauliflower and cabbage (pooled data, 2017-18 and 2018-19) at different blocks in Gurugram, Haryana

Locations (Blocks)	Cauliflower (mean/ 10 plants)		Cabbage (mean/ 10 plants)	
	P. xylostella	*S. litura*	*P. xylostella*	*S. litura*
Pataudi	10.90	5.03	7.20	4.88
Farukhnagar	6.03	4.25	5.23	3.30
Sohna	6.05	3.60	5.83	4.13
Gurugram	3.68	2.38	3.65	2.78
SEm±1	0.47	0.28	0.32	0.22
CD (p=0.05)	1.52	0.92	1.03	0.73

Table 2. Yield of cauliflower and cabbage along with benefit cost ratio obtained with biopesticides (pooled data)

Treatment	Dose (ml/ l)	No. of spray	Yield control (q/ ha)	Increased yield over control (Rs/ ha)	Price of product (Rs/ ha)	Cost of treatment (Rs/ ha)	Benefit of treatments (Rs/ ha)	Cost benefit ratio
Cauliflower								
Neem Azal	5	2	150.27	28.6	370.0	5025.0	14995	1:2.98
Neem Gold	5	2	163.77	42.1	350.0	4775.0	24695	1:5.17
Neemol	5	2	161.37	39.7	400.0	5400.0	22390	1:4.14
Nimbicidine	5	2	179.40	57.7	380.0	5150.0	35261	1:6.84
NLE	5	2	147.40	25.7	165.0	2462.0	15549	1:6.31
NSKE	5	2	168.13	46.46	215.0	3087.5	29435	1:9.53
Rakshak	2	2	154.03	32.36	367.0	4993.7	27341	1:5.47
Carbosulfan	2	2	198.80	77.13	700.0	6150.0	47841	1:7.71
Control	–	–	121.67	–	-	-	-	-
SEm±1	-	-	4.03					
CD (p=0.05)	-	-	12.20					
Cabbage								
Neem Azal	5	2	143.6	28.9	370.0	5025.0	14627	1:2.91
Neem Gold	5	2	157.1	42.4	350.0	4775.0	24057	1:5.03
Neemol	5	2	154.7	40.0	400.0	5400.0	21800	1:4.03
Nimbicidine	5	2	172.3	57.6	380.0	5150.0	34018	1:6.60
NLE	5	2	146.3	31.6	165.0	2462.0	19026	1:7.72
NSKE	5	2	162.0	47.3	215.0	3087.5	29076	1:9.41
Rakshak	2	2	151.3	36.6	367.0	4993.7	19894	1:3.98
Carbosulfan	2	2	188.7	74.0	700.0	6150.0	44170	1:7.18
Control	–	–	114.7	–	-	-	-	-
SEm±1	-	-	4.84					
CD (p=0.05)	-	-	14.66					

Labour charges Rs. 400/ day (2 No.); Machine charges Rs. 100/ day; Market price of cauliflower Rs. 700/ q; Market price of cabbage Rs.680/ q

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SEASONAL INCIDENCE OF MAJOR INSECT PESTS OF MUNGBEAN

Prince Mahore* and A K Pandey[1]

Department of Entomology, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior 474 002, Madhya Pradesh, India
[1]Department of Entomology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482 004, Madhya Pradesh, India
*Email: princemahore30@gmail.com (corresponding author): ORCID ID 0000-0003-0910-3236

ABSTRACT

A field experiment conducted during the kharif 2018 evaluated the seasonal incidence of insect pests of mungbean. The results revealed occurrence of major sucking insect pests viz., whitefly *Bemisia tabaci* (Genn.), leafhopper *Empoasca kerri* Pruthi, aphid *Aphis craccivora* Koch from vegetative stage to maturity stage. The lepidopterans viz., tobacco caterpillar *Spodoptera litura* (F.) and blue butterfly *Lampides boeticus* L., were observed from reproductive to maturity stage. Maximum incidence of *B. tabaci* was -at 9.70 nymphs and adults/ cage, *E. kerri* at 4.97 nymphs and adults/ plant, *A. craccivora* at 2.17 nymphs and adults/ plant, *S. litura* at 0.33 larvae/ plant and *L. boeticus* at 0.37 larvae/ plant and during the 39th standard week. Incidence of *B. tabaci, A. craccivora, S. litura* and *L. boeticus* showed significant positive correlation with maximum temperature, (r=0.79, 0.76, 0.82 and 0.85, respectively; *B. tabaci* with rainfall and *S. litura* with evening relative humidity showed a significant negative correlation (r=-0.76, -0.81, respectively).

Key words: Mung bean, sucking pests, lepidopteran pests, seasonal incidence, weather parameters, correlation coefficients, temperature, rainfall, relative humidity

Mung bean (*Vigna radiata* L. Wilczek) is an important pulse crop in India after chickpea and pigeon pea (Ved et al. 2008). It Hussain et al., 2011), and its productivity in India is 629 kg/ ha. It is also consumed as fresh sprout, seeds used for making soups, bread and biscuits (Sehrawat et al., 2013). The low productivity of mung bean in Madhya Pradesh may be attributed to a wide variety of factors, among which insects is of paramount importance. A number of insects have been recorded on mung bean, and In India, 64 species of insect pests reported (Lal, 2008). The present study evaluates the effects of weather parameters viz. max. temp.(°c), min. temp. (°c), sunshine (hrs), rainfall (mm), morning RH (%), evening RH (%), wind speed (km/ hr) and evaporation (mm) on the incidence of major sucking and lepidopteran insect pests in mung bean..

MATERIALS AND METHODS

The experiment was carried out on "Virat" variety sown at the breeder seed production unit, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India during kharif 2018-19. P in aplot size of 20x 10 m^2 was followed with a row to row and plant to plant spacing of 30 x 10 cm, and all agronomic practices were adopted except the pest control measures. Observations on insect pests were recorded from randomly selected 10 plants at weekly interval, starting from 15 DAS (days after sowing) and continued till the crop maturity. Sucking insect pests *Aphis craccivora* (Koch.) and *Empoasca kerri* Pruthi were observed on 3 compound leaves viz., top, middle and bottom; *Bemisia tabaci* (Genn.) population was recorded by using cage method. The lepidopterans *Spodoptera litura* (F.) and *Lampides boeticus* L., were recorded on 10 randomly selected plants by counting the number of larvae/ plant. Incidence of these pests was correlated with weather parameters; maximum and minimum temperature (°C), sunshine (hrs), rainfall (mm), morning and evening RH (%), wind speed (km/ hr) and evaporation (mm). Regression equations were developed for the ones with significant impact (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The major insects that attack during the vegetative stage (15 DAS i.e. 35 SMW) were *B. tabaci*, *A. craccivora* and *E. kerri*, and these infestations persisted up to maturity 41st SMW (Sahoo and Patnaik, 1994; Nath,1994; Singh and Kalra, 1995; and Dar et al., 2002 *S. litura* and *L. boeticus* were observed at reproductive stage, which were active till maturity; the former was observed from 36 DAS (38- 41 SMW) agreeing with earlier reports of Sujayanand et al. (2021); and the latter from 29 DAS (37 to 41 SMW). Irulandi and

Balasubramanian (1999), Ebadah (2002) and Sarkar et al. (2008) also studied seasonal incidence of insect pests of mungbean.

During kharif 2018, the population dynamics of these pests were recorded and correlated with weather factors. *Bemisia tabaci* appeared during 35th SMW (1.07 whiteflies/ plant) and remained active till maturity of the crop (4.20 whiteflies/ plant), with peak of 9.70 whiteflies/ plant being in 39th SMW. This observation corroborates with that of of Chandra et al. (2021). With *B. tabaci* a significant positive correlation (r=0.79) was observed with maximum temperature, while a significant negative correlation (r=-0.76) was observed with rainfall; regression equations being $\hat{Y}$=0.786x-18.841 and $\hat{Y}$=-0.03x+6.4866 respectively. These agree with those of Tamang et al. (2017). *E. kerri* appeared on on 35th SMW (0.40 adults/ plant), continued till 41th SMW with peak (2.17 adults/ plant) being *e*on 39th SMW. Yadav and Singh (2006) developed forecasting models for *E. kerri* and *B. tabaci*. *Aphis craccivora* appeared on the 35th SM, continued till 41st SMW, with a peak (4.97 aphids/ plant) on the 39th SMW; correlations revealed a significant positive correlation (r=0.76) with maximum temperature, and regression equation was $\hat{Y}$=0.4229x-10.203, agreeing with the findings of Bairwa and Singh (2017). *S. litura* appeared on the 38th SMW (0.17 larvae/ plant), continued till 41st SMW, with a peak (0.33 larvae/ plant) on the 39th SMW; its incidence showed a significant positive correlation (r=0.82) with maximum temperature, and a significant negative one (r=-0.81) was with evening RH, and the regression equations were $\hat{Y}$=0.04x-1.0887 and $\hat{Y}$=-0.0084x+0.7277, respectively. *Lampides boeticus* appeared on the 37th SMW, continued till 41st SMW with a peak of 0.37 larvae/ plant during the 39th SMW; a significant positive correlation (r=0.76) was observed with maximum temperature, and regression equation was $\hat{Y}$=0.0454x-1.2188 (Table 1, 2).

Table 1. Seasonal activity of insect pests of mung bean- Jabalpur (kharif, 2018-19)

SMW	Period of observations	Incidence				
		Sucking insect pests (Nymphs and adults)/ plant			Lepidopteran insect pests (larvae/ plant)	
		Bemisia tabaci	*Empoasca kerri*	*Aphis craccivora*	*Spodoptera litura*	*Lampides boeticus*
35	27 Aug-2 September	1.07	0.73	0.40	0.00	0.00
36	3-9 September	3.07	1.67	0.77	0.00	0.00
37	10-16 September	5.07	2.37	1.33	0.00	0.03
38	17-23 September	7.23	3.87	1.70	0.17	0.17
39	24-30 September	9.70	4.97	2.17	0.33	0.37
40	1-7 October	6.73	4.27	1.73	0.27	0.30
41	8-17 October	4.20	1.57	0.70	0.20	0.20

SMW= Standard Meteorological Week

Table 2. Correlation coefficients- incidence of insect pest vs. weather factors

Weather factors	Incidence of insect pests									
	B. tabaci (cage method)		*A. craccivora*/ plant)		*E. kerri*/ plant		*S. litura*/ plant		*L boeticus*/ plant	
	r	byx	r	byx	r	byx	r	byx	r	byx
Max. Temp.(°c)	0.79*	0.78	0.76*	0.42	0.74NS	–	0.82*	0.78	0.85*	0.79*
Min. Temp. (°c)	-0.13 NS		-0.07NS		0.02NS		-0.53NS		-0.50NS	
Sunshine (hrs)	0.72NS		0.65NS		0.67NS		0.70NS		0.73NS	
Rainfall (mm)	-0.76*	-0.03	-0.67NS		-0.69NS		-0.60NS		-0.62NS	
Morning RH (%)	-0.53NS		-0.42NS		-0.42NS		-0.67NS		-0.67NS	
Evening RH (%)	-0.73NS		-0.69NS		-0.66NS		-0.81*	-0.008	-0.83NS	
Wind speed (km/ hr)	-0.59NS		-0.56NS		-0.53NS		-0.75NS		-0.79NS	
Evaporation (mm)	0.72NS*		0.66NS		0.71NS		0.57NS		0.62NS	

r = Correlation; byx = Regression equation; NS-Non significant

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NEW RECORD OF THE MEDITERRANEAN RECLUSE SPIDER *LOXOSCELES RUFESCENS* (DUFOUR) FROM EASTERN INDIA

Somanath Sahoo*, Gitanjali Mishra, Jaya Kishor Seth, Laxman Kumar Murmu and Santhosh Goud

Post Graduate Department of Zoology, Berhampur University, Ganjam 760007, Odisha, India
*Email: cool.somanath@gmail.com: ORCID ID 0000-0002-0638-9497

ABSTRACT

A new record of *Loxosceles rufescens* (Dufour, 1820) (Araneae, Sicariidae) from Eastern India is discussed herein. This paper extends the distribution of *L. rufencens* from Western India to Eastern India. It was sampled from the wild habitat area inside the campus of Berhampur University, Ganjam, Odisha, India.

Key words: *Loxosceles rufescens*, Sicariidae, Mediterranean recluse spider, Eastern India, new record, invasive species, distribution, redescription

The family Sicariidae Keyserling (1880) of Order Araneae, includes spiders that are well known for their painful bites, which can cause necrotic severe skin damage at the biting site (Swanson and Vetter, 2006; Vetter and Isbister, 2008; Yigit et al., 2008). These spiders are easily distinguished by their six rather than eight eyes, which are grouped in three clusters of two eyes (ocelli) each. This family includes three genera, *Hexophthalma* Karsch, 1879, *Loxosceles* Heineken and Lowe, 1832 and *Sicarius* Walckenaer, 1847, having 8, 143 and 21 species worldwide, respectively (World Spider Catalog Version 23.0). Only one genus *Loxosceles* (violin spiders) has been documented in India, with a lone species, *Loxosceles rufescens* (Dufour, 1820), with distribution as various states of western India, including Gujarat, Jammu and Kashmir, Karnataka, Lakshadweep, Maharashtra, Manipur, Sikkim and Tamil Nadu (Trivedi and Dal, 2019; Tiwari et al., 2021). *Loxosceles rufescens* is commonly called the Mediterranean recluse spider, and originally found in the Mediterranean countries. This spider species has now spread to most countries, including Australia, the United States, East Asia, Africa, and China (Taucare-Rios et al., 2018). The major source of the invasion of this species is the transportation of goods by ship (Nentwig, 2017; Taucare-Rios et al., 2018). Its natural microhabitat consists of sparsely dispersed plants and beneath rocks and cracks (Nentwig et al., 2017). The invaded area's usual microhabitats are old buildings, tree barks and leaf litter (Borkan et al., 1995; Greene et al., 2009). It is both a passive hunter making irregular webs and an active hunter at night (Fischer et al., 2006). Its feeding habit is polyphagic as it can feed on a wide variety of diurnal and nocturnal insects, but in the invaded region, its preferred preys are woodlice, termites, ants, silver fishes and cockroaches (Greene et al., 2009). There are many publications regarding the severe and fatal bite of this species (Nentwig et al., 2017). Therefore, this species has medical importance, although there is no report of spider bites by this species in India.

MATERIALS AND METHODS

Spiders were sampled from wild habitats inside the campus of Berhampur University, Ganjam, Odisha, India (19˚17'56.62"N, 84˚53'01.26"E). Two techniques, beating and shaking of branches to the inverted umbrella and direct putting into the plastic vial, were used. If possible, the microhabitat of each spider was recorded and photographed in the field. Then the specimens were photographed in the laboratory using a DSLR camera with a 40 mm macrolens and preserved in 70% isopropyl alcohol in an air-tight labelled vial. The identification was done using key characters in Jocque and Dippenaar-Schoeman (2007) and Gertsch and Ennik (1983). One specimen of a female adult spider was identified to be *Loxosceles rufescens* (Dufour, 1820). The specimen was observed under a dissecting microscope, with large parts of the body measured using a divider and scale and smaller parts were measured with reference to the previously measured parts from perpendicularly taken macrophotographs. The genitalium was dissected and kept in 10% NaOH for 30 min and cleared using a fine brush and distilled water. Then the epigynum part was placed on the dry slide, and one drop of clove oil to observe under a stereozoom microscope (Levi, 1965). The voucher specimen is deposited and registered in

the National repository of EBRC-Zoological survey of India, Gopalpur-on-Sea, Odisha, India, with registration number: EBRC/ZSI/Ar13897.

RESULTS AND DISCUSSION

***Loxosceles rufescens* (Dufour, 1820)** (Fig. 1.A)

Material Examined: 1 example, Registration Number: EBRC/ZSI/In-12261 A-P, collected by: Somanath Sahoo, Adult: ♀ Medium-sized; body length 7.6 mm.

Redescription: Female: Total body length is 7.6 mm. Carapace is 3.3 mm long, 2.7 mm wide. The abdomen is 4.5 mm long and 3.2 mm wide. The Colour of the body is bright orange-brown. The lower parts of the legs are lighter than the femur. The entire body is covered with black hairy setae. Clypeus 0.15 mm long. Six eyes are present in three groups, one median group and two lateral groups (Fig. 1B). The space between median eyes and lateral eyes is 0.15 mm. Eyes are small and almost equal in size. The sternum is 1.6 mm long, 1.1 mm wide. The labium is 0.53 mm long. The chelicera is 0.92 mm long. The length of parts of appendages (legs, pedipalp) is as follows:

Appendages	I	II	III	IV	Palp
Femur	4.40	4.75	4.15	4.70	1.25
Patella	1.15	1.15	0.95	1.20	0.45
Tibia	4.55	4.85	3.70	4.30	0.85
Metatarsus	4.60	5.15	4.35	5.15	
Tarsus	1.20	1.30	1.15	1.25	1.25
Total	15.90	17.20	14.30	16.60	3.80

Leg formula (shortest as 1 to longest as 4) 2413. The second pair of legs is the longest, and the third pair of legs is the shortest. Epigynum (Fig. 1D) with genital furrow and swelled epigynum visible from the ventral side (Fig. 1C); receptacle closely located and produced into lobular sacs tilting to the inner side. Dark sclerotized bands along the outer face of the receptacles are present.

Ecology: The sampled spider was collected from the leaves of a wild shrub plant in the resting stage at 11.30 am. High trees entirely shaded the microhabitat. Gound was covered by leaf litter with moist soil due to rain one day before.

Potential threats: This new sighting of this species indicates that it can adapt to live in human habitat and

Fig. 1. *Loxosceles rufescens;* A. Dorsal view; B. Arragement of six eyes on cephalothorax; C. Ventral view showing female genitalia and spinneret; D. Dissected epigynum of genitalia

can easily relocate and establish itself in a new place to increase its population range throughout the world. As it is an invasive species, it can have the potential behaviour of dominance over native spider fauna to cause a drastic imbalance in the food chain in different microhabitats. Its bite can cause medical problems in children and hypersensitive groups of people. Therefore there is an immediate requirement to evaluate its relocation rate to a new place, establishment rate to increase its population size and ecological interference to native spider populations.

Remarks

Loxosceles rufescens, is now cosmopolitan and invasive in most countries. As the species possess ecological and medical importance, in some countries like USA, Brazil, Thailand etc., there are some guidelines to manage and treat the spider bite of the species by the local government (Nentwig et al., 2017). The new sighting of the spider *Loxosceles rufescens* from Odisha is the first documentation from Eastern India. The current record of its new distribution provides preliminary information about its faster invasion rate and potential threat to the spider fauna in India. This species may inflict necrotic severe skin damage at the biting site. The current record of its expanding distribution provides information about its rapid invasion rate and potential threat to the spider fauna in India.

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AUTHORS' CONTRIBUTIONS

S Sahoo conducted the sampling, collected, took the photographs and identified the specimens. S Sahoo, G Mishra, J K Seth, L K Murmu and S Goud prepared the manuscript. All authors read and approved the manuscript.

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COMPATIBILITY OF ENTOMOPATHOGENIC FUNGUS *METARHIZIUM RILEYI* WITH BIORATIONALS

Kajal Bharti, Neelam Joshi*, Saijal Khosla and Rabinder Kaur[1]

Department of Microbiology; [1]Department of Entomology,
Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: neelamjoshi_01@pau.edu (corresponding author): ORCID ID 0000-0002-4159-5691

ABSTRACT

In this study, *Metarhizium rileyi* isolates (*M. rileyi* NIPHM, *M. rileyi* MTCC 4254 and *M. rileyi* MTCC 10395) were evaluated for their compatibility with other entomopathogenic fungi viz. *Metarhizium anisopliae* NBAIR (Ma-35), commercial formulation of *M. anisopliae*, *M. anisopliae* (Local), *Beauveria bassiana* ITCC 7126, *B. bassiana* (Local) and commercial formulation of *Verticillium lecanii* and neem based formulations- (azadirachtin 0.03%EC) and (azadirachtin 0.15%EC) along with synthetic insecticide spinetoram 11.7%SC. Neem-based formulations viz., azadirachtin 0.03% EC 1.5 ml/ l and 0.15%EC 5 ml/ l reduced *M. rileyi* MTCC 4254 growth by 44.77% and 53.73% over control, respectively; *M. rileyi* MTCC 4254 was more compatible with *M. anisopliae* NBAIR (Ma-35) and commercial formulation of *M. anisopliae* with less reduction in growth (24.3% and 24.9%, respectively); but it was least compatible with commercial formulation of *V. lecanii*. *M. rileyi* MTCC 4254 recorded 71.64% reduction with spinetoram 11.7% SC (0.4 ml/ l). Thus, *M. rileyi* was compatible with *M. anisopliae* NBAIR (Ma-35), *M. anisopliae* followed by azadirachtin 0.03% EC, and these could be used as components in IPM.

Key words: *Metarhizium rileyi*, *M. anisopliae*, *Beauveria bassiana*, *Verticillium lecanii*, azadirachtin, spinetoram, compatibility, reduction in growth, IPM

Entomopathogens are microbial control agents used for crop pest management. Entomopathogenic fungi (EPF) like *Metarhizium anisopliae*, *Beauveria bassiana, Verticillium lecanii* and *Metarhizium rileyi* are widely used against pest in agriculture fields and greenhouses. These are ecofriendly and have an important role in plant protection for sustainable IPM (Grewal and Joshi, 2021). Synthetic pesticides traditionally used have negative effects, and efforts have been made to reduce their use. *Metarhizium rileyi* (Farlow) Kepler, Rehner and Humber, formerly known as *Nomuraea rileyi* (Kepler et al., 2014), is a fungus with a specific host range that is used as a biocontrol agent for the management of lepidopteran pests. These secrete secondary metabolites that act as immunosuppressive compounds which lead to fungal infection (Constanza et al., 2019). Botanicals can also be used as an alternative because they pose little risk to humans and they can be readily combined with many other bioagents (Mohan et al., 2007). Azadirachtin is less harmful to the environment and prevents development of insect resistance (Isman, 2006). In IPM the compatibility of such fungi with botanicals and pesticides is very important (Neves et al., 2001) and can improve effectiveness with less pollution risks by reducing the amount of pesticides used (Usha et al., 2014). Compatibility studies on these are important to deploy such biopesticides and biocontrol agents (Rashid et al., 2010). Therefore, compatibility of entomopathogenic fungi with botanicals/ other microbials is necessary (Sahayaraj et al., 2011). This study assesses the compatibility of *M. rileyi* with neem formulations, insecticides and other entomopathogenic fungi.

MATERIALS AND METHODS

Two *M. rileyi* isolates, viz. MTCC 10395 and MTCC 4254, were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India, and one isolate, *M. rileyi* NIPHM, was procured from the National Institute of Plant Health Management, Hyderabad, India. These isolates were grown and maintained on Sabouraud maltose agar with yeast extract (SMAY) (mycological peptone 1%, maltose 4%, agar 2%, yeast extract 1% and chloramphenicol 0.5%), and refrigerated till further use. Compatibility study of *M. rileyi* with azadirachtin and insecticide was carried out using the poison food technique with some modifications (Reddy et al., 2021). Azadirachtin 0.03%EC (@ 1.5 ml/ l, @ 2.5 ml/ l), azadirachtin 0.15% EC (@ 5 ml/ l, @ 10 ml/ l) and spinetoram 11.7%SC (@ 0.2 ml/ l, @ 0.4 ml/ l) were added individually to

the sterilized growth culture media and poured into the petri plate after proper agitation and allowed to solidify. This supplemented growth media plate was inoculated with 1 mm fungal disc of fully-grown *M. rileyi.* A control plate with a pure culture of *M. rileyi* was used. These plates were incubated at 25±2°C for ten days. *M. rileyi* compatibility studies with entomopathogenic fungi were carried out using the dual culture technique with some modifications (Sumalatha et al., 2017). The autoclaved media was poured into petri plates and allowed to cool. After solidification the plates were inoculated with *M. rileyi* at one side of the plates and test entomopathogenic fungi was kept at opposite side in individual plates. A pure culture of *M. rileyi* on growth media was used as control. These plates were incubated at a 25± 2°C for ten days, and % inhibition was recorded. Data given represent the mean and± SD (standard deviation) by applying one-way ANOVA done in SPSS 16.0 statistical software.

RESULTS AND DISCUSSION

Ten days after incubation, *M. rileyi* MTCC 4254 recorded maximum radial growth (3.7± 0.14) with 44.77% growth reduction over control on SMAY media supplemented with azadirachtin 0.03%EC at 1.5 ml/ l concentration (Table 1); *M. rileyi* MTCC 10395 recorded minimum radial growth (2.2± 0.14) and maximum growth % reduction (62.71%) over control when media was supplemented with azadirachtin 0.15%EC (10 ml/ l) concentration. Results showed that azadirachtin 0.03%EC at lower concentration (1.5 ml/ l) was safe and compatible with *M. rileyi*. This may be due to the effect of concentrations of neem derivatives on the growth of *Metarhizium* spp. Hirose et al. (2001) also observed that neem derivatives containing <5% or neem oil containing <0.25% were less toxic to *M. anisopliae* mycelial growth and spores. Neem oil at lower concentrations is compatible and synergistic when combined with entomopathogenic fungi such as *B. bassiana* and *Metarhizium* spp. However, beyond such concentrations, spore viability or colony growth is delayed and suppressed, indicating that neem oil can cause loss of potency or inhibition of entomopathogenic agents (Togbé et al., 2014). Dev et al. (2021) evaluated *M. rileyi* Farlow (Samson) impregnated with azadirachtin against *Helicoverpa armigera* (Hubner) and reported *M. rileyi* blended with azadirachtin @ 10^6 conidia/ ml and @ 10^8 conidia/ ml caused the highest mortality of 86.21% and 89.66% of 2nd and 3rd instar larvae of *H. armigera,* respectively. Sahayaraj et al. (2011) used liquid and dual plate bioassays to investigate the compatibility of commercial botanicals (Biospark, Exodos, and Phytophrate) with *Isaria fumosorosea*, *Beauveria bassiana* and *Lecanicillium lecanii* in vitro. They found that commercial botanicals significantly reduced the mycelial growth of *B. bassiana*, *L. lecanii* and *I. fumosorosea*. Mohan et al. (2007) screened 30 isolates of *Beauveria bassiana* for compatibility with commercial formulation of neem oil (Margoside®) at field recommended dose (0.3% v/v) and reported 23 isolates were compatible with neem. In neem sensitive isolates growth was decreased but not totally inhibited.

In vitro compatibility study of *M. rileyi* with insecticide spinetoram 11.7% SC recorded 71.64% growth reduction over control in *M. rileyi* MTCC 4254 (Fig. 1). Variation in radial growth, growth % reduction over control and sporulation of *M. rileyi* (Farlow) Samson was recorded by Matcha et al. (2021). They reported that fungicides carbendazime,

Table 1. *M. rileyi* growth on SMAY supplemented with azadirachtin @ 0.03% and 0.15% EC)

Treatment	Control**	Conc. of azadirachtin @ 0.03% EC (ml/ l)	*M. rileyi* growth on SMAY supplemented with (azadirachtin @ 0.03% EC) (cm) (Mean± S.D)* (after 10 incubation)		Conc. of azadirachtin @ 0.15% EC (ml/ l)	*M. rileyi* growth on SMAY supplemented with Indo neem (azadirachtin @ 0.15% EC) (cm) (Mean± S.D)* (After ten days incubation)	
			Radial growth	Growth % reduction over control		Radial growth	Growth % reduction over control
M. rileyi NIPHM	6.4± 0.14	1.5	3.3± 0.07	48.43	5	2.9± 0.42	54.68
	6.4± 0.14	2.5	3.2± 0.21	50.00	10	2.7± 0.70	57.81
M. rileyi MTCC 4254	6.7± 0.21	1.5	3.7± 0.14	44.77	5	3.1± 0.56	53.73
	6.7± 0.21	2.5	3.5± 0.42	47.76	10	2.95± 0.21	55.97
M. rileyi MTCC 10395	5.9± 0.42	1.5	2.9± 0.56	50.84	5	2.35± 0.49	60.16
	5.9± 0.42	2.5	2.8± 0.56	52.54	10	2.2± 0.14	62.71

*Values mean± SD; **Control- Growth media without supplementation of azadirachtin

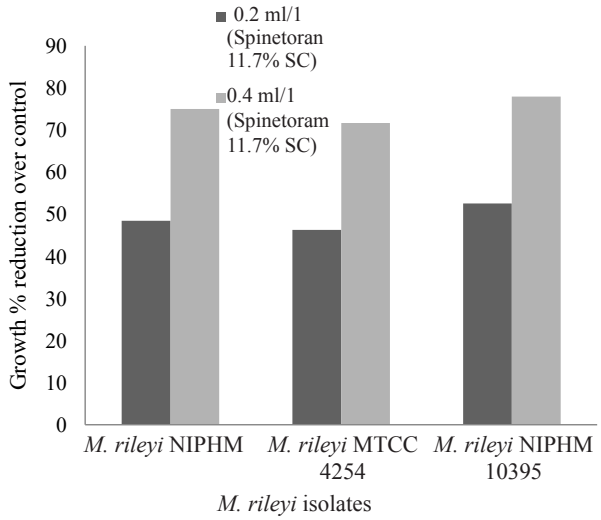

Fig. 1. Growth % reduction of *M. rileyi* with spinetoram

propiconazole showed complete growth inhibition (100%), insecticide Emamectin benzoate 5 SG showed a maximum inhibition of 77.37% while Azoxystrobin and Tebuconazole recorded lower growth inhibition of 8.17% and 12.36% respectively. The compatibility studies of *Metarhizium* (*Nomuraea*) *rileyi* rice bran oil formulation with four insecticides recorded higher % inhibition of 34.67 by novaluron and lower % inhibition of 23.56 was recorded by lufenuron (Saheb et al., 2021). The difference in radial growth or % inhibition observed could be attributed to the inherent variability of chemicals to biological agents. This could be due to the inconsistent interaction between fungus and insecticides. Compatibility study of *M. rileyi* with other entomopathogenic fungal strains revealed that *M. anisopliae* NBAIR (Ma-35) recorded maximum radial growth (5.9± 0.36) with minimum growth % reduction (24.3%) over control followed by the commercial formulation of *M. anisopliae* with 24.9% growth reduction over control against *M. rileyi* MTCC 4254. However, it was least compatible with *V. lecanii* (Fig. 2). This variation in compatibility may be due to difference in fungal growth parameters. Limited published information is available regarding the compatibility of *M. rileyi* with other entomopathogenic fungi to substantiate the complete results of the present study. The current study concluded that neem formulation, (azadirachtin 0.03% EC) at lower dose 1.5 ml/ l was safe for *M. rileyi*. Further, *M. rileyi* was more compatible with *M. anisopliae* and was least compatible with commercial formulation of *V. lecanii*. However, further field studies will enable better understanding of compatibility studies, which can lead to the use of these formulations for ecofriendly IPM.

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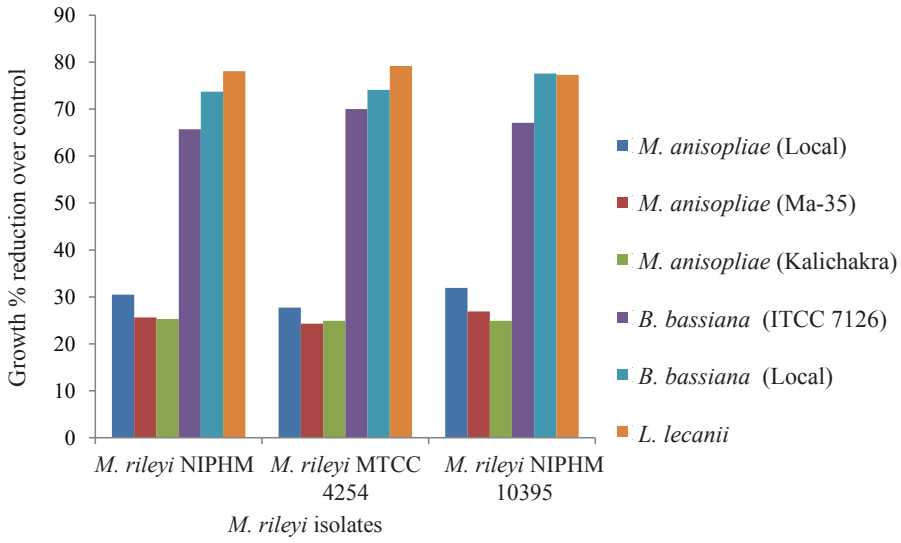

Fig. 2. Growth % reduction of *M. rileyi* with entomopathogenic fungi

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INSECTICIDAL ACTIVITY OF ESSENTIAL OILS FROM MINT AND AJWAIN AGAINST PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* (L)

Grishma Pipariya, Pankaj Sharma, Nishi Pandya and Pragna Parikh*

Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, Gujarat, India
*Email: Php59@yahoo.co.in (corresponding author): ORCID ID 0000-0001-7990-3517

ABSTRACT

The present study evaluates the insecticidal activity of two essential Oil (EOs) mint (*Mentha arvensis*), and ajwain (*Carum capyicum*) against pulse beetle (*Callosobruchus chinensis)* (L). Contact toxicities of these were evaluated using parameters of lifecycle like total development period, numbers of eggs laid, adult emergence and adult longevity. Along with these detoxification enzyme inhibition activities of acetyl cholinesterase (AChE), alkaline phosphatase (ALP), transaminases enzymes- aspartate aminotransferases (AST) and alanine aminotransferases (ALT) and total protein were estimated. EOs were observed showing toxicity (mint LC_{50} = 5.9 µl/ ml and ajwain LC_{50}= 7.02 µl/ ml). Exposure of EOs altered the lifecycle parameters significantly ($p<0.01$). The detoxification enzyme inhibition activities were also significant ($p<0.01$). Thus, it is concluded that these EOs can be recommended as safe and ecofriendly alternatives.

Key words: *Callosobruchus chinensis*, essential oils, *Mentha arvensis*, *Carum capyicum*, lifecycle, acetyl cholinesterase, alkaline phosphatase, transaminases enzymes, inhibition

India is one of the leading producers of food in the world and it produces more than a billion tonnes of agricultural product. 58% of India's population is dependent on agriculture as its primary source of livelihood. In India, advancement of technology has increased the production of grains; however, improper storage has resulted in huge loss and has been reported to be around INR 926 billion loss annually (Singh and Khanna, 2019; Sirohi et al., 2021). Infestation of stored grain by many insects, mite and fungi degrade the quality and quantity of grains (Lal et al., 2017; Jerbi et al., 2021). The total productivity of agricultural crops of India is 3 tonnes/ha; out of which loss due to insect pest is about 26 % (Lal et al., 2017), like the lesser grain borer, *R. dominica*'s larva and adult infests the grains and declines its quality (Jerbi et al., 2021). Rice pest *S. oryzae*, causes qualitative and quantitative loss (Saad et al., 2018). *C. chinensis* a major pest of stored pulses and is reported to cause 32-64% loss under storage condition (Femeena et al., 2018). After discovery of DDT, Insect pests are mainly controlled by synthetic pesticides (Lal et al., 2017; Demeter et al., 2021). WHO has reported that every year two lakhs people die due to pesticide poisoning owing to its carcinogenic and teratogenic properties (Sarwar, 2016). Use of synthetic pesticide is a easy and quick solution for controlling insect pests but pose a potential risk not only to humans but also to the environment as their residues have been reported to be present in soil, air and water (Said and Pashte., 2015; Lal et al., 2017). The repeated uses of synthetic insecticide for decades has disrupted biological control by natural enemies and has led to outbreaks of other insect species and at times have resulted in resistance of pesticides in insect pest (Hill et al., 2017; Hawkins et al., 2019). Hence, there is need for alternative solution which environment friendly does not harm other non-target species. Plants and their derivatives have been proved to be a viable alternative as more than 2000 plant species have been recorded to possess insecticidal properties and possess low health risks (Pavela, 2016; Jerbi et al., 2021). EOs are naturally produced by plants as secondary compounds which are volatile, but as natural products protects the stored grains from pest attack (Omar, 2020). EOs has multiple components mixture and causes toxicity by interfering with various aspects of insect's physiology and biochemistry (Kiran et al., 2017). Present work evaluates the insecticidal potential of the two EOs *M. arvensis* and *C. capyicum* against *C. chinensis* (pulse beetle) adults.

MATERIAL AND METHODS

The adult insects were collected from the infested grains from the granary and were reared on 500 g green gram (variety - Sabarmati PS 16) maintained in laboratory at Department of Zoology, The Maharaja Sayajirao University of Baroda. A culture of *C. chinensis*

was maintained on green grams, the legume seeds were washed with hot water to remove any pesticide residues, dried under sun to eliminate any infestation. These seeds were stored in air tight plastic container until required for experiments, which were carried out at a constant temperature of 28± 2ºC, relative humidity 70± 5% at 16:8 light: dark photoperiod (Bhumi et al., 2017). Morphological Identification of insect pest was done by using standard keys (Raina, 1970).

C. chinensis were categorized into two groups: Group I: Control (Acetone treated). Group II: EOs exposed test group, which were again subdivided into Low dose (LD) and High dose (HD) following the standard toxicological guidelines of OECD. To determine the lethal concentration (LC_{50}) value of EOs, serial dilutions (0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 1, 1.5, 2, and 2.5) µl/ml in acetone as solvent were prepared. 1 ml of each concentration was uniformly applied a glass petridish of diameter 15cm x 2cm; after evaporation of acetone (10 minutes), 10 pairs of adult were placed in each petridish. Same procedure was used for the control treated with acetone. Mortality was recorded after interval of every 24 hours till 96 hours. (Bhumi et al., 2017). After obtaining LC_{50} value, 10 pairs of adult *C. chinensis* in 50gms of green grams were exposed to the LD and HD of mint and ajwain for seven days on and on the eight day they were sacrificed and tissue were pooled for the further analysis. The green grams with the eggs were separated and on every 24hrs interval eggs were observed for the egg incubation period, larval development period, pupation period. Day of adult emergence was recorded followed by total number of adult emerges and longevity of the emerged adults.

For performing biochemical assays, homogenate was prepared for which individual adult insects were separately weight & homogenized in 10 volumes (w/v) of ice cold 0.1 M phosphate buffer (pH 7.2 - AChE, AST and ALT and total protein) and for ALP the homogenate was made at 9.8 pH using glass homogenizer. Homogenates were centrifuged at 5000 rpm for 30 min at 4ºC. The obtained supernants were divided into small portion & stored at -20ºC. Ache Activity was determined according to Ellman et al. (1961), specific activity was expressed as mmol/ml/min. Alkaline Phosphatase Activity was determined according to Klin (1972), specific activity was expressed as IU/ L. Transaminases Activity was determined according to Reitman and Frankel (1957), specific activities were expressed as IU/ L. Total soluble protein was determined calorimetrically according to the method of Lowry et al. (1951) by using bovine serum albumin (BSA) as a standard. Total protein expressed as g/ dl. The difference in the life cycle parameters and detoxification enzyme activities of control and test groups were determined by One-Way ANOVA using Graph pad Prism software v.8.

RESULTS AND DISCUSSION

The results revealed that the LC_{50} value of the EOs of mint is 5.9 µl/ ml and that of ajwain is 7.02 µl/ ml; $1/5^{th}$ and $1/ 20^{th}$ concentration were selected as low dose and high dose from the obtained LC_{50} with the pulse beetle *Callosbruchus chinensis*; other values were- (M) LD (0.29 µl/ ml) and (M) HD (1.18 µl/ ml) while (A) LD (0.35 µl/ ml) and (A) HD (1.40 µl/ ml). A dose dependent variation was observed in the parameters of lifecycle- significant reduction in eggs laid, adult emergence and adult longevity was observed ($p<0.01$). High dose of ajwain gave more significant alteration compared to mint (Fig.1). Campolo et al. (2018) suggested that application of EOs act as oviposition deterrent, and impacts the overall lifecycle. Perez et al. (2010) reported a dose dependent mortality on exposure of *A. inulifolium* oil to *S. granarius.* A dose dependent alteration in the enzyme activities of AChE, ALP, AST and ALT was also observed. Both the EOs significantly inhibited the enzyme activities compared to control ($p<0.01$) (Fig. 2). Similar effect was also noticed with total protein. Thymol, a terpenoid is major constituent of ajwain oil (67.4%) and is reported to have an insecticidal effect due to its aromatic ring and hydroxyl group (Vitali et al., 2016). Singla et al. (2018), opined that the conversion of thymol to thymyl acetate and thymyl phenylether inhibits the enzyme activity. Fumigant action of thymol results into interdicting plasma membrane lipids altering the membrane potential leading to loss of metabolites and ions from the cells (Chaudhari et al., 2021). Main constituents of mint oil have been well explored (Vendan et al., 2017), and menthol, a volatile molecule had been shown to possess fumigant and repellency properties against *C. maculatus* and *Sitophilus oryzae* (Vendan et al., 2017; Saeidi et al., 2017)

In inhibition of the AChE activities observed now in in accordance with previous work on *M. piperita* EOs compounds, against *S. oryzae* and *T. castaneum* (Rajkumar et al*.,* 2019). EOs from cumin and basil have been reported to inhibit the ALP activity in *S. oryzae* (Omar*,* 2020). In the present study, a significant reduction in the ALP at high dose of ajwain was

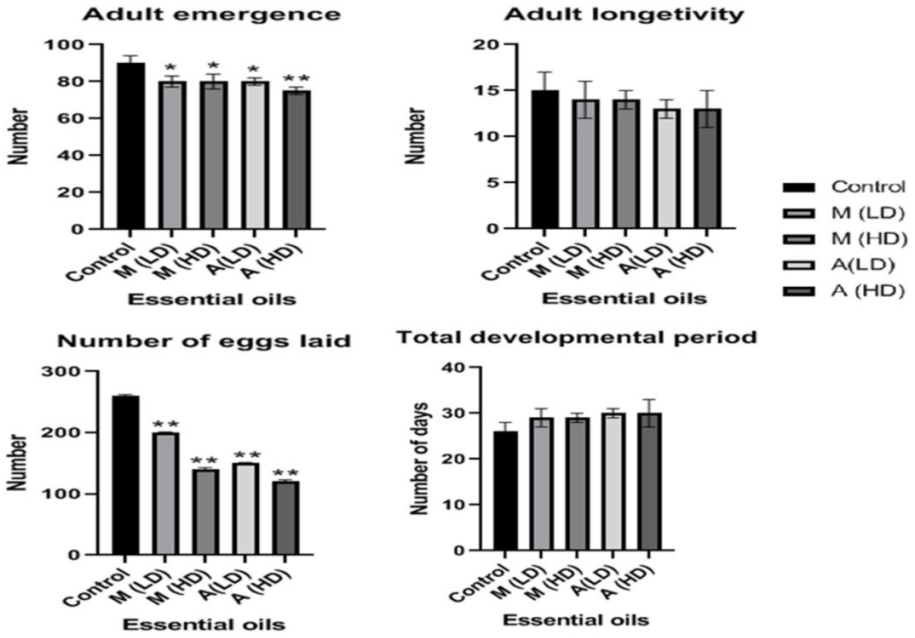

Fig. 1. Effect of EOs on adult emergence, longevity, eggs laid and development period *(p<0.05); ** (p<0.01)

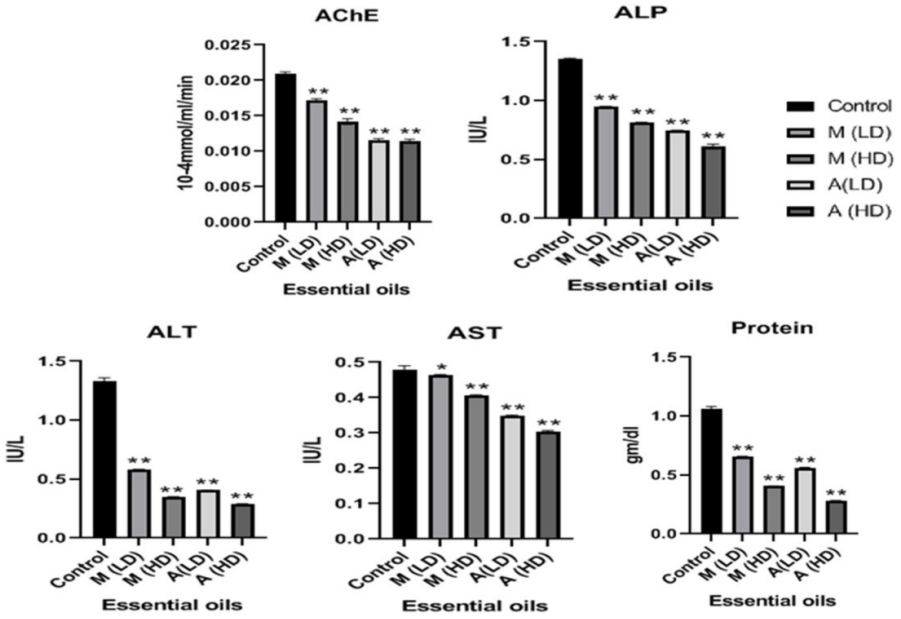

Fig. 2. Inhibitory effects of EOs on the enzymes- AChE, ALP, AST, ALT and total protein *(p<0.05); ** (p<0.01)

observed. It is probably due to reduction of phosphorous liberated for energy metabolism, decreased rate of metabolism, as well as decreased rate of transport of metabolites (El-Gizawy et al., 2019; Omar, 2020). ALT as well as AST both serves as a strategic link between the carbohydrates and protein metabolism known to be altered during various physiological and pathological conditions (Tawfeek et al., 2021) Various EOs have been reported to alter AST and ALT activities (Shahriari et al., 2017; Kisa et al., 2018; Hashem et al., 2020). The intrinsic properties of EOs interfere with basic metabolic, biochemical, and physiological functions of insect pests. The two EOs evaluated cause significant decrease in total protein content which may be attributed to reduced protein synthesis and low uptake of amino acids (Omar et al., 2020). Ayalew (2020) and Tawfeek et al. (2021) also observed a decrease in total protein content in *S. oryzae*. Thus, it can be concluded that mint

and ajwain EOs can be recommended as ecofriendly alternatives.

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AUTHOR CONTRIBUTION STATEMENT

GP, PS, NP, PP designed and conceptualized the study. GP performed the experimental work. GP and PS analyzed the data. GP, PS and NP, wrote the draft manuscript. PS, NP, PP reviewed and revised the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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POPULATION DYNAMICS OF RICE YELLOW STEM BORER AND SHEATH BLIGHT

Rakesh Kumar Sharma* and K K Sharma[1]

Farm Advisory Service Centre (Punjab Agricultural University), Gangian 144205, Punjab, India
[1]Regional Research Station (Punjab Agricultural University), Ballowal Saunkhri,
Balachaur 144521, Punjab, India
*Email: rksharma@pau.edu (corresponding author)

ABSTRACT

Field experiments were conducted on the seasonal incidence of yellow stem borer (*Scirpophaga incertulas*) and sheath blight (*Rhizoctonia solani*). These revealed that maximum deadheart incidence (5.32 and 5.64%) was observed in the 33rd and 34th standard meteorological week (SMW) in 2018 and 2019, respectively. The incidence of white earhead was maximum (4.96 and 5.28%) during 40th SMW. Maximum incidence of sheath blight (50 and 46.67%) was noticed during 36th SMW. A significant positive correlation of minimum temperature and evening relative humidity was found with deadheart incidence. Conversely, maximum temperature and morning relative humidity had significant positive correlation with incidence of sheath blight. The model developed for ascertaining effect of weather factors on incidence of pest and disease showed a good relationship between predicted and observed data of % deadheart (R^2=0.996), white earhead (R^2=0.992) and diseased plants (R^2=0.986).

Key words: Rice, *Scirpophaga incertulas*, Rhizoctonia blight, correlation, deadheart, white earhead, regression models, temperature, relative humidity

Rice (*Oryza sativa* L.) is cultivated in tropical and subtropical countries including India and Punjab occupies an area of 31.42 lakh ha with total production of 189.18 lakh mt and productivity of 60.21 q/ ha (Anonymous, 2021). Occurrence of insect pests and diseases is the major limiting factor in rice productivity in India. Among the 23 species of insects attacking rice (Atwal and Dhaliwal, 2005; Pasalu and Katti, 2006), yellow stem borer (*Scirpophagam incertulas*) causes deadheart and white earheads (Sulagitti et al., 2018) causing direct yield losses (Rahman et al., 2004). In addition to this, sheath blight incited by *Rhizoctonia solani* Kuhn has also emerged an important threat in all rice growing areas with 25 to 50% yield losses (Roy, 1993; Prasanna Kumar and Veerabhadraswamy, 2014; Shinde and Prashanthi, 2014). Precise agroecosystem information on incidence and distribution of pest and disease in relation to weather parameters is prerequisite to develop any management programme (Patel and Shekh, 2006; Singh et al., 2012). Many studies have been conducted to study the effect of various weather parameters on seasonal abundance of yellow stem borer, their population buildup and progression of sheath blight (Rana et al., 2017; Nag et al., 2018; Seni and Naik, 2018; Shilpa et al., 2018, Bisen et al., 2019, Jasrotia et al., 2019). During last decade there has been an increase in area under rice cultivation in lower Shiwaliks of Punjab and there are frequent queries by the farmers regarding incidence of *S.incertulas*) and *R.solani*. The present study was conducted to find out the peak period congenial for the incidence and progression of aforesaid pest and disease on rice in different locations in lower Shiwalik area of Punjab, India and its relation with various weather factors.

MATERIALS AND METHODS

A survey of paddy fields (Variety PR 126) in the vicinity of village Saunkhri of Balachaur block of Punjab, India was undertaken from July to October, 2018 and 2019 during kharif season to record weekly data on incidence of *S. incertulas* and *R. solani*) from three randomly selected plots (1 m^2 area) of three farmer's fields following standard procedure. The incidence of *S. incertulas* was assessed by counting number of deadhearts (DH) and white earheads (WEH) in vegetative and reproductive stage, respectively. Incidence of *R. solani* was recorded by counting number infected plants in random sample. The weather parameters, viz., maximum temperature, minimum temperature, average relative humidity and rainfall (data obtained from meteorology observatory at Dr Dev Raj Bhumbla Regional Research Station, Ballowal Saunkhri) were correlated with the incidence and correlation coefficients were worked out. Regression models for predicting the incidence were worked out

for temperature (T_{max} and T_{min}), relative humidity (RH_{mor} and RH_{eve}) and rainfall (RF), in XLSTAT software using regression technique. The two years (2018 and 2019) recorded data was divided in a ratio of 70:30 for model development (70 and 30% for model development and validation, respectively). The performance analysis of developed regression models included computation of different statistical parameters viz. mean absolute % error (MAE), root mean square error (RMSE) (Ramanathan, 1995), standard deviation (SD) and Willmott index of agreement (d) (Willmott et al., 2012).

RESULTS AND DISCUSSION

During kharif 2018 and 2019, the incidence level of *S. incertulas* was medium to low; deadhearts start appeared during 3rd week of July reaching maximum level of 5.32 % during 2nd week of August 2018 (33rd SMW) and 5.64% during 3rd week of August 2019 (34th SMW) (Fig. 1). Similarly white earhead incidence started to appear during 1st week (0.42%) and 2nd week of September (0.42%) during 2018 and 2019, respectively; maximum level of 4.96 and 5.28% were during 1st week of October (40th SMW) which start decreasing thereafter. These observations corroborate with those of Murali et al. (2017) that deadheart incidence started during 1st week of August 31st SMW which reached at peak during 3rd week and 4th week of August. The present findings are also similar to those of Kalita et al. (2020). Correlation coefficients revealed a positive and significant values with minimum temperature (r=+0.57 & +0.48) and evening RH (r=+0.49 & +0.46), respectively; maximum (r=-0.01 & -0.14) and minimum temperature (r=-0.28 & -0.34), morning (r=-0.34 & -0.16) and evening RH (r=-0.31 & -0.29), rainfall (r=-0.41 & -0.29) were negatively but non-significantly correlated with white earhead during 2018 and 2019, respectively; while a significant negative correlation was observed with number of rainy days (r= -0.45* & -0.59*). Minimum temperature and evening relative humidity enhanced the deadheart incidence. Murali et al. (2017) observed that relative humidity had positive correlation with deadhearts while it was negative for white earhead incidence with temperature, RH and rainfall. These results corroborate with those of Pallavi et al. (2018) and Sawai and Kothikar (2019), Kumar et al. (2020). Patel and Singh (2017) reported that weather parameters are the major ones with incidence of *S. incertulas*. Weekly observations from 27th to 46nd SMW, on incidence of *R. solani* revealed a continuous increase from 34th to 36th SMW reaching 50 and 46.67% during 36th SMW of 2018 and 2019, respectively (Fig. 1). Correlation coefficients revealed that a maximum temperature around 32-34 °C and >90% RH conducive. These results are accordance with those of earlier findings (Pasalu et al., 2005; Bhukal et al., 2015; Nandi, 1980; Tiwari and Chaure, 1997; Biswas et al., 2011; Bhukal et al., 2015). A significant positive correlation of maximum temperature and morning RH with incidence was evident (Fig. 1). Lenka et al. (2008) observed a significant positive correlation with incidence.

The statistical comparison indicated that the developed models were sufficiently accurate to predict the deadheart, white earhead and diseased plants in relation to the recorded T_{max}, T_{min}, RH_{mor}, RH_{eve},

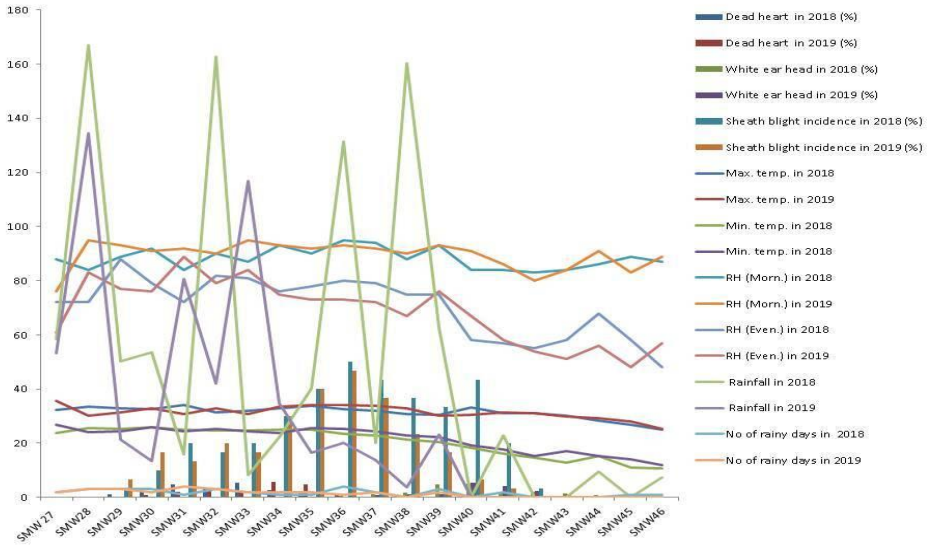

Fig. 1. Population dynamics of *S. incertulas* and *R. solani* of rice (kharif 2018, 2019)

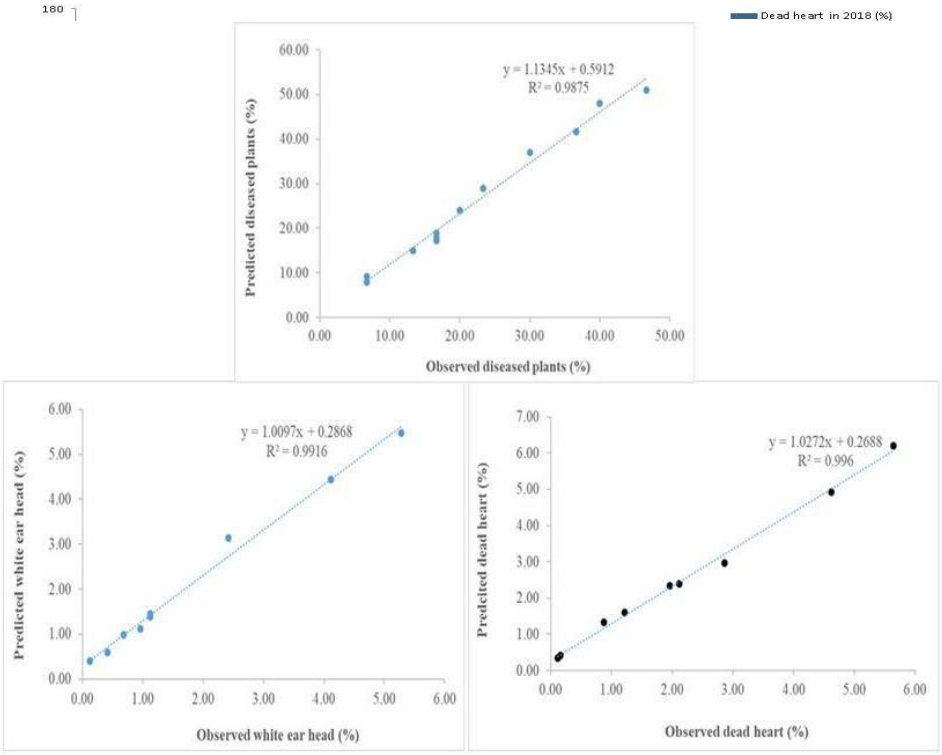

Fig. 2. Model (observed and predicted % deadheart, white earheads and diseased plants)

and RF. The predicted % deadheart formed a good relationship with the observed values for year 2019 as indicated in Fig. 2 (R^2=0.996). Similarly, good relation was recorded between predicted and observed data of white earhead and % diseased plants. The statistical parameters viz. MAE, RMSE, SD and d values were computed to be in the range of 0.33-3.66, 0.35-4.32, 0.13-2.30 and 0.98-1.00, respectively. Both predicted and observed data indicated a high degree of agreement. The present results are in accordance with Chander and Palta (2010), who analyzed location- specific relations between pest and weather using empirical models and Manibhushanrao and Krishnan (1991) who also formulated a simulation model (EPIBLA) for leaf Blast disease of rice using multiple regression equations based on maximum temperature and maximum RH.

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AUTHOR CONTRIBUTION STATEMENT

RKS and KKS conceived and designed research experiments. RKS and KKS conducted experiments. RKS and KKS analyzed data. RKS and KKS wrote the manuscript. Both authors read and approved the manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest or competing interests.

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INSECT PESTS OF KHASI MANDARIN IN EAST AND WEST KHASI HILLS DISTRICT OF MEGHALAYA

Krishna Kumar S*, P Balasubramanian and Penumajii Ganesh Kumar

Department of Entomology, School of Crop Protection (SCP), College of Post Graduate Studies in Agricultural Sciences (CPGS-AS), Central Agricultural University (CAU, Imphal), Umiam (Barapani) 793103, Ri-Bhoi District, Meghalaya, India
*Email: sikibharathi@gmail.com (corresponding author)

ABSTRACT

Roving survey of insect pests on Khasi mandarin carried out during August 2018 to February 2019 in the East and West Khasi hills districts of Meghalaya revealed the spectrum of insect pests. The survey covered five villages in each district, that is major citrus growing one. The results revealed eight insect species as the more dominant, and incidence observed individually for these showed that the citrus leaf miner (*Phyllocnistis citrella*) was found in abundantly in all the villages. The vector pests of citrus aphid (*Toxoptera citricida*) was found more in citrus tristeza viral disease prone areas and citrus blackfly (*Aleurocanthus woglumi*) also occurred.

Key words: Meghalaya, East and West Khasi hills, Khasi mandarin, Meghalaya, vectors, pests, tristeza, *Phyllocnistis citrella, Aleurocanthus woglumi,* dominants'

Citrus is native to the north eastern India and adjacent valley which is collectively called as Indo-Burma region. GI tagged Khasi mandarin is one of the major and seasonal fruits of Meghalaya, and East and West Khasi hills districts are major producers. Decline of Khasi mandarin is governed by several factors which result in less productivity in Meghalaya (4.95 t/ ha) compared to India's average (11.08 t/ ha) (Anonymous, 2017). Among different factors, is the visible damage caused by insects, and hence details of their incidence needs to be recorded. Since, the crop becomes susceptible to pests due to its variable climate as well as unattended and uncared plantations these details are of utmost importance. In the north eastern region around 42 insect species are found assuming as major and minor pest status in the mandarin causing citrus decline (Hore and Barua, 2004). Among them the citrus leaf miner (*Phyllocnistis citrella* Stainton) and lemon butterfly (*Papilo demoleus* L.) are common. Citrus trunk borer (*Anoplophora versteegi* Ritzema) can make the whole tree to fall down. Sucking pests such as aphids (*Toxoptera citricida* Kirkaldy) have vector importance; blackfly (*Aleurocanthus woglumi* Ashby), mealybug (*Planococcus citri* Risso) and psylla (*Diaphorina citri* Kuwayama) cause severe citrus decline (Sreedevi, 2010). Hence this study to evaluate the emerging pest complex of Khasi mandarin in the state of Meghalaya particularly in East and West Khasi hills districts.

MATERIALS AND METHODS

Roving survey was carried out in different Khasi mandarin growing villages in East and West Khasi hills districts (EKH& WKH) to assess the infestation level of insect pests. In each district, five villages were selected i.e, Mawryngkneng, Sohryngkham, Mawlynnong, Pynursla, Nohwet (EKH) and Mawphanniew, Kynrud, Sohpi, Mairang, Nongshillong (WKH) Among all villages five orchards each having 25 randomly selected trees had been chosen and the observations of insect damage were recorded. The method of survey mainly employed for various insects are, citrus leaf miner by number of mining leaves in total number of leaves (% infestation), larval population per tree were recorded for lemon butterfly, for citrus trunk borer boreholes were counted, citrus psylla were recorded by number of nymphs in 5 cm length of twig, the vector pest citrus aphid were recorded by counting the number of insects per square centimeter, citrus black fly has been recorded number of insects present in randomly selected 15 leaves/ tree and citrus mealybug were recorded by number of nymphs in 5 cm length of twig per tree (Rao et al., 2012).

RESULTS AND DISCUSSION

Citrus leaf miner *Phyllocnistis citrella* was found in all the villages surveyed, with maximum (56%) infestation being at Kynrud (WKH) and minimum by

(38.7%) in Mawlynnong (EKH), and it is a predominant pest (Fig. 1, 3). The incidence level was > the ETL of 25% infected leaves, affecting at least 10 leaves/ tree. Singh (1984) observed this on younger and tender leaves, which became distorted and get curled, with irregular twisted galleries or zigzag glistering tunnels on both sides of the leaves. It frequently affects the stems leading to loss of vigour (Pena et al., 2000). For the lemon butterfly *Papilio demoleus* larval counts ranged from 1.1 to 1.5/ tree (Fig. 2, 4); it was noticed on tender and older leaves, with its ETL of 3-5 larvae/ tree not exceeded, making it as a minor pest. Mahesh and Pizvi (2003) reported total defoliation due to *P. demoleus*, while Raju and Naidu (2000) observed *Citrus aurantium* (lemon tree) as its preferred host for its egg laying. Citrus trunk borer *Anoplophora versteegi* incidence was observed as 0.6 to 1.0 boreholes/ tree (Fig. 2, 4); and among the villages surveyed three villages namely, Sohryngkham (EKH), Sohpi (WKH) and Mairang (WKH) were found to show incidence above the ETL (1 borehole/ tree); and West Khasi hills are affected more than East Khasi hills. Citrus trunk borer infestation is quite important due to its severe form of damage on trunk. Singh and Singh (2012) observed its grub boring into the trunk at ground level horizontally up to the pith and then tunnelling vertically and then again return to horizontal to exit. Infected trunks exude gum and wood powder accumulation on ground, with tree succumbing at short period.

Citrus psylla *Diaphorina citri* affected the tree in all six villages with maximum incidence of 0.9/ 5cm twig (Fig. 2, 4) being at Sohryngkham (EKH). In West Khasi hills the khasi mandarin plants were found free from this except one village Kynrud. Nymphs were less in the villages surveyed. Citrus aphid *Toxoptera citricida* population was found to occur in seven villages, and at Mawryngkneng (EKH) it was significantly more (13.9/ cm^2 twig) followed by Nohwet (EKH) (Fig. 2,5), and its ETL is 5-8/ cm^2 twig). Thus, it predominantly occurred in East Khasi hills. Symptoms of citrus tristeza viral disease were observed herein. In addition to viral transmission, its infestation is attributed for stunted and reduction of fruit setting (Ghosh et al., 2015). Citrus blackfly *Aleurocanthus woglumi* was found @ 18.4/ leaf in Pynursla (EKH), and it was also observed at Mawphanniew (WKH), Nongshillong (WKH) and Mairang (WKH) (Fig. 2, 5) above ETL (5-10 nymphs per leaf). This pest infects by sucking sap from underside of the leaf resulting in yellowing, stunting and reduction in yield. Chatterjee et al. (2000) observed yield loss of fruits up to 50-70% due to this pest. Citrus

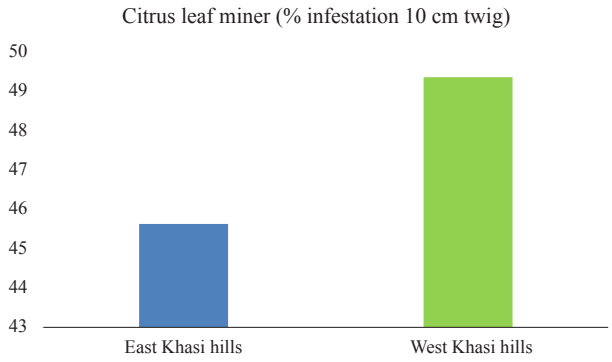

Fig. 1. Citrus leaf miner infestation

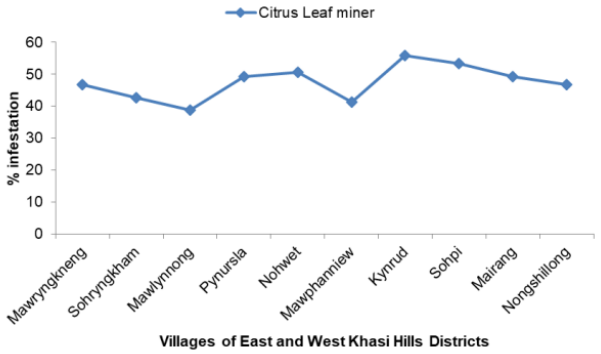

Fig. 2. Lemon butterfly, trunk borer, psylla, blackfly, mealybug

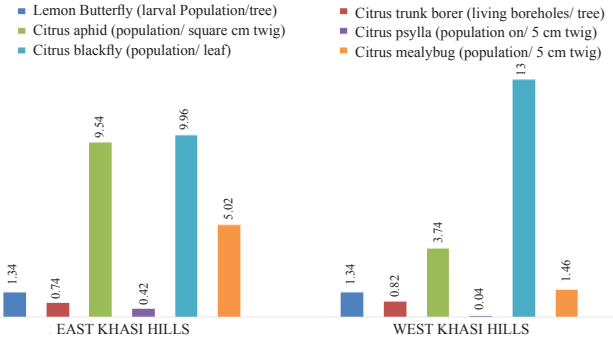

Fig. 3. Leaf miner (10 cm twig) infestation

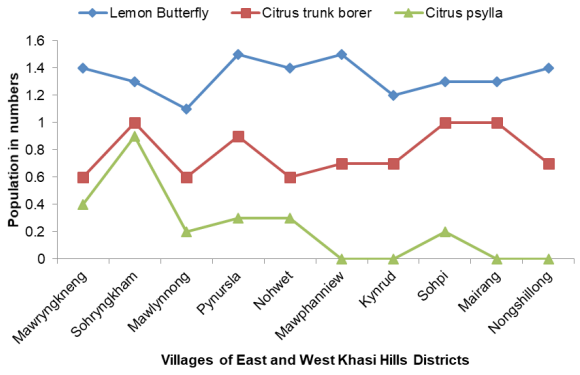

Fig. 4. Lemon butterfly, trunk borer, citrus psylla infestation

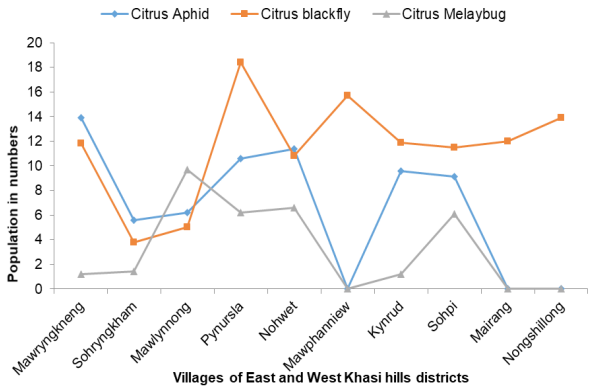

Fig. 5. Citrus aphid, blackfly, and mealybug

mealybug *Planococcus citri* occurred in seven villages, with a maximum of 9.7/ 5 cm twig in Mawlynnong (EKH) which was followed by 6.6 numbers/5 cm twig in Nohwet (EKH) and 6.2 numbers/5cm twig in Pynursla (EKH). In West Khasi hills only two villages namely, Sohpi and Kynrud were found affected and remaining villages found free from pest (Fig. 2 & Fig. 5). Citrus mealybug reduces the yield by their colonisation occurrence. It was very destructive at fruiting stage. Similar form of damage symptom was identified in the observation made in the five villages and found with density exceeding ETL (3-5 nymphs per 5 cm twig). Rao et al., (2012) reported its sporadic nature, prefers humid conditions planted on heavier soils or with large and/or closely planted trees.

Finally, the roving survey indicated that lacking intercultural operations such as pruning of old, withered and dried twigs leads to low yield of mandarin. These affected plantations should be rejuvenated by undertaking integrated pest and nematode management programme from the data on economic threshold level (ETL) collection from time to time, stage to stage, field to field on prevailing climatological and the other biotic and abiotic factors. Although, the government should take initiative to extension programmes in different blocks to educate the khasi mandarin growers and farmers to maximize its production as it will make the Khasi mandarin much valuable GI product of Meghalaya.

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EFFICACY OF INSECTICIDES AGAINST AMERICAN SERPENTINE LEAF MINER *LIRIOMYZA TRIFOLII* (BURGESS) IN WATER MELON

Sankar C*, Jayaraj J, Shanthi M, Chinniah C, Vellaikumar S[1] and Rajamanickam C[2]

Department of Agricultural Entomology; [1]Department of Biotechnology,
Agricultural College and Research Institute,
Tamil Nadu Agricultural University (TNAU), Madurai 625104, Tamil Nadu, India
[2]Citrus Research Station, TNAU, Vannikonenthal 627 951, Trinelveli, Tamil Nadu, India
*Email: csankarento@gmail.com (corresponding author)

ABSTRACT

A field experiment was conducted to evaluate the efficacy of certain selective insecticides against the serpentine leaf miner *Liriomyza trifolii* (Burgess) in water melon *Citrullus lanatus* (Thunb.) Matsum and Nakai. The hybrid F1 (Melody) was sown in protray and 13 days old seedlings were transplanted in the main field. Significant less live mines (1.48/ leaf) was observed with chlorantraniliprole 18.5%SC @0.6 ml/ l. This was followed by flubendiamide 39.35%SC @0.6 ml/ l (2.09/ leaf) and indoxacarb 14.5%SC @0.65 ml/ l (3.05/ leaf). Leaf damage/ plot was 2.72, 3.48 and 4.03%, with chlorantraniliprole, flubendiamide and indoxacarb, respectively. Significantly maximum yield of fruits (25.5 t/ ha) was obtained with chlorantraniliprole given as three sprays at 15 days interval. Thus, chlorantraniliprole 18.5%SC @0.6 ml/ l can be recommended against *L. trifolii* in water melon.

Key words: Water melon, kharif season, farmer field, Namakkal district, *Liriomyza trifolii* leaf damage, chlorantraniliprole, flubendiamide, indoxacarb, infestation reduction, fruit yield, ICBR

Water melon is an important commercial horticultural crop rich in vitamins A, B_1, B_2 and C and minerals (Moniruzzaman, 1988). China is the largest producer and India occupies third position (Anonymous, 2019). Tamil Nadu has an area of 6.420 ha with water melon and with productivity of 32 t/ ha (Santosh et al., 2018), and the cultivation is restricted to Villupuram, Namakkal, Ariyalur, Coimbatore and Erode districts (Chadha, 2013). More than 35 varieties/ hybrids are grown in India. It is attacked by several insect pests at various stages (Anonymous, 2012). Serpentine leaf miner *Liriomyza trifolii* is its most destructive pest in its early growth stage. It causes loss of 15-70 % in French bean, 41% in cucumber and 35% in tomato (Krishna Kumar, 1998), and in water melon, maximum leaf damage (37%) has been observed (Patnaik, 2000). Apart from causing direct losses, it also causes wounds on the plant foliage and predisposes it to secondary infection by bacterial and fungal pathogens. Farmers resort to applying several rounds of insecticides that are harmful to human beings and environment (Anonymous, 1991). This study evaluates certain newer insecticides effective at lower doses against *L. trifolii* in water melon under field conditions.

MATERIALS AND METHODS

The field experiment was carried out at farmer's field at Muthur village (11° 6'17"N, 78° 6'7"E), Namakkal district of Tamil Nadu during kharif 2019. The selected insecticides were compared with farmers practice of foliar spray of profenophos 50%EC@ 2ml/ l. The insecticides evaluated include- T_1-Thiamethoxam 25%WG@ 0.4g/ l; T_2-Imidacloprid 17.8%SL@ 0.3 ml/ l; T_3-Spiromesifen 22.9%SC@ 0.5 ml/ l; T_4-Diafenthiuron 50%WP@ 0.8 g/ l; T_5-Thiacloprid 21.7%SC@ 0.6 ml/ l; T_6-Propargite 57%EC@ 1.25 ml/ l; T_7-Chlorantraniliprole 18.5%SC@ 0.6 ml/ l; T_8-Flubendiamide 39.35%SC@ 0.6 ml/ l; T_9-Indoxacarb 14.5% SC@ 0.65 ml/ l; T_{10}-Fenazaquin 10% EC@ 2 ml/ l; T_{11}-Chlorfenapyr 10% EC@ 1 ml/ l; T_{12}-Malathion 50% EC (Treated check) @ 1 ml/ l; T_{13}-Untreated check (water spray) @500 l/ ha. Three replications were maintained with popular hybrid F_1 (Melody) sown in protray and 13 days old seedlings transplanted in the main field at a spacing of 2.5 x 0.5m and other recommended package of practices were adopted. The first spray was done with the onset of pest incidence after recording pretreatment count of leaf miner and subsequent ones repeated after 15 days interval using high volume sprayer. The post-treatment counts were recorded on 1, 3, 7, 14 days after spray. Ten plants were selected randomly from each replication and the infested live mines were recorded from 3 leaves/ creeping branches (one from unopened leaves and two

opened leaves) and the infestation level was assessed. The observations on % leaf damage and score were done using the sampling grade as follows (% infestation, category of intensity: 0-10, Very low; 11-20, Low; 21-30 Moderate; 31-40 Severe; and >41 Very Severe The observation on leaf damage (%) was converted as score values as given by Galande (2001) and Onkara Naik et al. (2019). Water melon fruits were harvested and pooled to arrive at the total fruit yield (t/ ha). The increase in yield and income over untreated check was worked out and the benefit cost ratio was calculated following the procedure- BCR = Gross income / (total cost of cultivation + cost of plant protection) and (cost of insecticide + labour charges for spraying) as adopted by Akila and Sundara Babu (1994). The data were analyzed for ANOVA. The data on incidence were transformed into square root transformation and analyzed in SPSS (version 22) (IBM Crop. Released 2013) software to identify the most effective treatments and their means were compared by significant difference at $p<0.05$ ANOVA following Tukeys' Honest Significant Difference test.

RESULTS AND DISCUSSION

The results showed that all the insecticides were effective in reducing the *L. trifolii* incidence. The data on number of live mines/ leaf, % leaf damage, % reduction of live mines over untreated check, % reduction leaf damage and increase in yield are given in Table 1. The results revealed a significant less number of live mines with chlorantraniliprole (1.48/ leaf) followed by flubendiamide (2.09/ leaf), indoxacarb (3.05/ leaf), as against maximum in the untreated check (12.83/ leaf). The % leaf damaged was the least in chlorantraniliprole treated plants (2.72%) followed by flubendiamide (3.48%), indoxacarb (4.03%) as against maximum in the untreated plot (35.31%). The % reduction in leaf damage over untreated check was the highest (92.29%) with chlorantraniliprole followed by flubendiamide (90.14%) and indoxacarb (88.59%) when sprayed at 15 days interval. A significantly high fruit yield (25.50 t/ ha) and incremental cost benefit ratio (ICBR) (1:1.56) was obtained with chlorantraniliprole followed by flubendiamide (24.43 t/ ha; 1:1.49), indoxacarb (23.50 t/ ha; 1:1.44) as compared to the untreated check of 16.30 t/ ha).

Variya and Patel (2012) reported the efficacy of diafenthiuron, emamectin benzoate, thiamethoxam and spinosad in reducing the leaf miner incidence and increasing the yield of water melon. Radhakrishnan and Natarajan (2009) also reported a significant effect of trap + dimethoate 30 EC@2 ml/ l and methyl demeton 25EC@ 2 ml/ l which registered lesser leaf miner incidence. Present results are in conformity with those of Saad Mousa et al. (2013) on the efficacy of chlorantraniliprole, chlorfenapyr, indoxacarb, and spinosad mixed with abamectin. Selvaraj et al. (2017) confirmed that chlorantraniliprole 4.3%+ abamectin 1.7% dose was significantly effective, while Sapkal et al. (2018) and Anjali et al. (2018) found that use of chlorantraniliprole, flubendiamide and indoxacarb is effective in tomato. Rohit et al. (2020) observed that during summer (2019), cyantraniliprole led to the least leaf mines (4.34%) and leaf damage (13.01%). Ramesh and Ukey (2007) observed the superiority of chlorantraniliprole and abamectin in tomato while Kousika et al. (2015) observed that chlorantraniliprole 4.3% + abamectin 1.7% SC mixture was effective against *Tuta abosoluta* (Meyrick) damage in tomato. Hafsi et al.(2012) and Braham et al.(2012) also obtained similar results. The fact that chlorantraniliprole, spinosad and chlorfenapyr are comparable in their efficacy was also observed by Smitha et al (2017), Connroy et al. (2008), Pereira et al (2014) and Naeem et al. (2016). Thus, it can be concluded that chlorantraniliprole is superior giving maximum fruit yield (25.50 t/ ha) and ICBR (1:1.56).

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Table 1. Evaluation of certain insecticides against *L. trifolii* in water melon

Treatments details	Mean no. of live mines / leaf (PTC)	Mean no. of live mines / leaf **(X± SE)*** **	Mean of % leaf damage/ plot **(X± SE)*** **	% reduction of live mines over untreated check	% reduction of leaf damage over untreated check	Category of intensity	****Fruit yield (t/ ha)	% increasing yield over untreated check (t/ ha)	ICBR
T -Thiamethoxam 25% WG @0.4g/l 1	10.65 (3.26)	3.33 (1.83)± **0.77^{c}**	6.65 (2.58)± 2.29^{cd}	73.69 (8.58)^{b}	81.17 (9.01)^{cd}	Very low	19.93 (4.46)^{def}	22.26 (4.72)^{f}	1:1.23
T -Imidacloprid 17.8% SL @ 0.3ml/ l 2	12.53 (3.54)	4.26 (2.06)± **0.78^{d}**	7.70 (2.77)± 2.33^{de}	66.35 (8.15)^{c}	78.20 (8.84)^{cd}	Very low	18.50 (4.30)^{fg}	13.49 (3.67)^{h}	1:1.13
T -Spiromesifen 22.9% SC @ 0.5ml/ l 3	12.25 (3.50)	3.23 (1.80)± **0.82^{c}**	6.11 (2.47)± 2.37^{e}	74.48 (8.63)^{b}	82.80 (9.10)^{bc}	Very low	22.93 (4.79) ^{bc}	21.93 (4.68)^{f}	1:1.14
T -Diafenthiuron 50% WP @ 0.8g/ l 4	10.25 (3.20)	4.19 (2.05)± **0.89^{d}**	7.57 (2.75)± 2.40^{cde}	66.9 (8.18)^{c}	78.57 (8.86)^{cd}	Very low	19.60 (4.43)^{efg}	20.24 (4.5)^{fg}	1:1.20
T -Thiacloprid 21.7% SC@ 0.6ml/ l 5	11.92 (3.45)	3.09 (1.76)± **0.91^{c}**	6.77 (2.60)± 2.43^{cde}	75.59 (8.69)^{b}	80.82 (8.99)^{cd}	Very low	23.03 (4.80) ^{bc}	41.28 (6.42)^{c}	1:1.29
T -Propargite 57% EC @ 1.25ml/ l 6	13.25 (3.64)	4.30 (2.07)± **0.92^{d}**	6.74 (2.60)± 2.45^{cd}	66.11 (8.13)^{cd}	80.91 (8.99)^{e}	Very low	21.50 (4.64)^{cd}	31.90 (5.65)^{d}	1:1.31
T Chlorantraniliprole18.5%SC@0.6ml/ l 7	10.13 (3.18)	1.48 (1.22)± **0.93^{a}**	2.72 (1.65)± 2.47^{a}	88.30 (9.4)^{a}	92.29 (9.61)^{a}	Very low	25.50 (5.05)^{a}	56.44 (7.51)^{a}	1:1.56
T -Flubendiamide39.35%SC@0.6 ml/ l 8	13.85 (3.72)	2.09 (1.44)± **0.92^{b}**	3.48 (1.86)± 2.48^{ab}	83.57 (9.14)^{a}	90.14 (9.49)^{a}	Very low	24.43 (4.94)^{ab}	49.87 (7.06)^{b}	1:1.49
T -Indoxacarb 14.5% SC@ 0.65ml/ l 9	12.67 (3.56)	3.05 (1.75)± **0.93^{c}**	4.03 (2.01)± 2.50^{b}	75.90 (8.71)^{b}	88.59 (9.41) ^{ab}	Very low	23.50 (4.85)^{b}	44.17 (6.65)^{c}	1:1.44
T -Fenazaquin 10% EC@ 2ml/ l 10	11.53 (3.40)	5.39 (2.32)± **0.94^{f}**	6.89 (2.62)± 2.51^{cde}	57.42 (7.58)^{e}	80.48 (8.97^{cd}	Very low	19.50 (4.42)^{efg}	19.63 (4.43)^{g}	1:1.19
T -Chlorfenapyr 10% EC@ 1ml/ l 11	10.13 (3.18)	4.88 (2.21)± **0.94^{e}**	6.79 (2.61)± 2.53^{cde}	61.45 (7.84)^{de}	80.78 (8.99)^{cd}	Very low	20.40 (4.52)^{de}	25.15 (5.01)^{e}	1:1.25
T -Malathion 50% EC @1ml/ l 12	13.62 (3.69)	4.10 (2.02)± **0.93^{d}**	8.24 (2.87)± 2.53^{e}	67.61 (8.22)^{c}	76.67 (8.76)^{c}	Very low	18.16 (4.26)^{g}	11.41 (3.38)^{i}	1:1.14
T -Untreated check -water@ 500 l / ha 13	13.92 (3.73)	12.83 (3.58)± **0.93^{g}**	35.31 (5.94)± 2.53^{f}	-	-	Severe	16.30 (4.04)^{h}	-	-
F	-	640.54	354.94	1609.82	770.15	-	1731.63	1225.09	-
P	*NS	**<0.000	**<0.000	**<0.000	**<0.000	-	**<0.023	**<0.000	-
SD	-	2.77	8.26	21.57	1.75	-	2.72	16.37	-
SE	-	0.77	2.29	5.98	0.51	-	1.34	4.54	-

PTC- Pre treatment count, *NS – Non significant, F= F value of Tukeys Test, P=statistically significance, ICBR-Incremental cost benefit ratio; SE- Standard Error, **Highly significant, SD-Standard deviation, **** Sale price of watermelon fruit was Rs.5.00 per kg, *** Each value is the mean of three replications; Figures in parentheses square root transformed values.; In a column, means followed by common letter(s) not significantly different (Tukey HSDs test at $_{P}$=0.05%)

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EVALUATION OF INSECTICIDES AGAINST LEPIDOPTERAN PESTS OF OKRA

M M Patel, R K Sharma, J R Patel[1] and Manishkumar J Joshi[2]*

Agricultural Research Station, S D Agricultural University, Ladol 382840, Gujarat, India
[1]Polytechnic in Agriculture, S D Agricultural University, Khedbrahma 383275, Gujarat, India
[2]Department of Agricultural Entomology, S D Agricultural University,
Sardarkrushinagar 385506, Gujarat, India
*Email: mj1107006@gmail.com (corresponding author)

ABSTRACT

Field experiment for the evaluation of insecticides was conducted at the Agricultural Research Station, S D Agricultural University, Ladol during kharif 2018- 2020. Results revealed that least fruit damage caused by *Earias vitella* F (9.84%) and *Helicoverpa armigera* Hübner (12.86%) was observed with emamectin benzoate 5SG at 0.0025%. Emamectin benzoate 5SG at 0.0025% gave maximum fruit yield and incremental cost benefit ratio. The pesticide residue analysis with emamectin benzoate 5SG at 0.0025% showed that the residues were below limit at 5 days after the spray. Also, only below MRL residue (0.223 ppm) of spinosad 45SC at 0.0169% was observed.

Key words: Okra, pests, *Helicoverpa armigera*, *Earias vitella*, emamectin benzoate, spinosad, yield, pesticide residue analysis, MRL, yield, cost benefits

Okra *Abelmoschus esculentus* L. Moench belongs to the family Malvaceae is grown in 509 thousand ha in India with a production of 6095 thousand mt (Anonymous, 2018). The cultivation of okra has many constraints, of which losses due to insect pests attack is important (Jagtab et al., 2007). As many as 72 pests are known on okra (Srinivasa and Rajendran, 2003). Among them, shoot and fruit borer, *Earias vitella* (F.) and fruit borer *Helicoverpa armigera* (Hubner) are major pests (Shitole and Patel, 2009), and cause >50% loss (Archunan et al., 2018). Insecticides are still largely in use against these pests. Conventional insecticides like endosulfan (Shivalingaswamy et al., 2008; Rath and Mukherjee, 2009), malathion and hostothion (Kumar and Gill, 2010),chlorpyriphos (Kuttalam et al., 2008), phosalone and quinalphos (Anonymous, 2011), and fenvalerate, methomyl, azinphos methyl, carbaryl and pyrethrin/ rotenone (Anonymous, 2012) had been used in management of lepidopteran pests on vegetable crops. In recent times, some new insecticide molecules offer multiple advantages in terms of greater levels of safety, better performance and reduced environmental impact (Visnupriya and Muthukrishnan, 2020). Therefore, this study was undertaken with the objectives to investigate the persistence toxicity of some novel insecticides against *E. vitella* and *H. armigera* under field conditions in okra.

MATERIALS AND METHODS

Field evaluation of insecticides was conducted at the Agricultural Research Station, S D Agricultural University, Ladol (23.638460° N, 72.705492° E) during kharif- 2018, 2019 and 2020. The variety Gujarat Anand Okra-5 was planted following all the agronomic practices as per package of practices for vegetables crops. The experiment was laid out in randomized block design (RBD) with eleven treatments including untreated control. Each treatment was replicated thrice. The treatments include: emamectin benzoate 5SG @ 250 ml (T1); spinosad 45SC @ 112.5, 150 and 187.5 ml (T2, T3 and T4, respectively); thiodicarb 75WP @ 375, 500 and 625 ml (T5, T6 and T7, respectively); novaluron 10EC @ 562.5, 750 and 937.5 ml/ ha (T8, T9 and T10, respectively); and untreated control (T11). For sprays 500 l of water/ ha was used with manually operated knapsack sprayer. Before each spray five plants were selected randomly/ plot and observations on *E. vitella* and *H. armigera* were made from selected plants. Two sprays were given at 15 days interval starting from initiation of damage. Fruit damage by *H. armigera* and *E. vitella* at harvest was recorded by counting total and damaged fruits before and after each spray. Yield data on different pickings was used to work out cumulative yield/ plot and converted to ha basis. Incremental cost benefit ratio of treatments was also worked out. Pesticide residue analysis was done for the two effective treatments, emamectin benzoate 5SG 0.0025% and spinosad 45SC 0.0169% at BioScience Research Centre, SDAU, Sardarkrushinagar using QUECHERs method. Thirteen fresh fruit samples of

okra (including control) were collected after the last (2nd) spray of insecticides at 0 day (2 hrs after the spray), 1, 3, 5, 7 and 10 days after the spray. All data were statistically subjected to ANOVA through SPSS Computer program (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) Significance of differences between the treatment means were compared by DNMRT at $p \leq 0.05$.

RESULTS AND DISCUSSION

Data on fruit damage (%) by *H. armigera* and *E. vitella* summarized in Table 1 show non-significant differences among treatments before spray. Data on fruit damage (%) at harvest after both the sprays and pooled analysis were found significant during kharif 2018, 2019 and 2020. Pooled mean of all three years revealed significantly least fruit damage (12.86%) with emamectin benzoate 5SG, 0.0025% and it was found significantly superior against *H. armigera*. Emamectin benzoate inhibits muscle contraction, causing a continuous flow of chlorine ions in the GABA and H-Glutamate receptor sites (Fanigliulo et al., 2009). The present results corroborate with those of Murugraj et al. (2006) on emamectin benzoate with larval population of *H. armigera.* Kanna et al. (2005) reported superiority of emamectin benzoate over lamda-cyhalothrin and spinosad against *H. armigera*. Fanigliulo et al. (2009) compared emamectin benzoate with spinosad and indoxacarb, and observed good control of *H. armigera* by emamectin benzoate. Emamectin benzoate is more potent on *Spodoptera littoralis* than lufenuron or spinosad (El Sayed and El-Sheikh, 2015). Data on fruit damage (%) by *E. vitella* at harvest after both the sprays and pooled analysis were found significant in kharif 2018, 2019 and 2020. Data on pooled mean revealed significantly the least total fruit damage (9.84%) with emamectin benzoate 5SG at 0.0025% at par with spinosad 45SC at 0.0169% (13.43%).

These results agree with those of Dhaker et al. (2017)

Table 1. Efficacy and economics of insecticides against *H. armigera* and *E. vitella* in okra

S. No.	Treatments	Fruit damage (%)				Yield	Gross realization (Rs.)	Cost of inputs	Net realization (Rs.)	BCR
		H. armigera		*E. vitella*						
		Before spray	After two spray	Before spray	After two spray					
T_1	Emamectin benzoate 5SG 0.0025%, 5 g	29.18 (24.05) *	20.89^{g} (12.86)	25.90 (19.17)	18.18^{c} (9.84)	14444^{a}	173333	47330	126003	2.66
T_2	Spinosad 45SC 0.0101%, 2.25ml	29.75 (24.65)	24.58^{ef} (17.40)	25.83 (19.14)	25.07^{ab} (18.02)	12865^{b}	154378	49405	104973	2.12
T_3	Spinosad 45SC 0.0135%, 3 ml	29.56 (24.63)	23.87^{ef} (16.47)	25.95 (19.46)	23.29^{ab} (15.73)	12930^{b}	155157	50830	104327	2.05
T_4	Spinosad 45SC 0.0169%, 3.75ml	29.58 (24.39)	22.87^{f} (15.18)	25.64 (18.83)	21.38^{bc} (13.43)	14030^{a}	168357	52255	116102	2.22
T_5	Thiodicarb 75WP 0.0563%, 7.5g	27.94 (22.11)	27.60^{bcd} (21.68)	25.96 (19.32)	24.31^{ab} (17.30)	12009^{bc}	144103	47980	96123	2.00
T_6	Thiodicarb 75WP 0.0750%, 10 g	28.66 (23.12)	26.61^{cd} (20.20)	26.21 (19.79)	24.91^{ab} (17.83)	12259^{b}	147104	48930	98174	2.01
T_7	Thiodicarb75WP, 0.0938%, 12.5g	30.02 (25.39)	25.75^{de} (19.08)	26.27 (19.78)	24.53^{ab} (17.26)	12407^{b}	148889	49880	99009	1.98
T_8	Novaluron 10EC 0.0113%, 11.25ml	30.07 (25.15)	29.42^{b} (24.25)	25.50 (18.70)	25.50^{ab} (18.62)	11048^{c}	132574	48730	83844	1.72
T_9	Novaluron 10EC 0.0150%, 15 ml	30.55 (26.35)	29.15^{b} (23.84)	26.59 (20.25)	25.23^{ab} (18.18)	11165^{c}	133979	49930	84049	1.68
T_{10}	Novaluron 10EC 0.0188%,18.75ml	29.54 (24.74)	28.39^{bc} (22.66)	25.88 (19.33)	25.60^{ab} (18.70)	11138^{c}	133656	51130	82526	1.61
T_{11}	Untreated Control	30.60 (25.98)	31.96^{a} (28.11)	28.48 (22.84)	27.75^{a} (21.72)	8864^{d}	106363	44250	62113	1.40
S. Em (±)		1.20	0.623	1.12	0.538	349				
CD (p=0.05)		NS	1.744	NS	1.506	983				
CV (%)		13.86	9.99	14.66	9.59	9.64				
Y x T		NS	NS	NS	NS	NS				

*Figures in parentheses arc sin transformed values; Means with letter(s) in common not significant by DNMRT (p=0.05); Emamectin benzoate 5SG 0.0025% (4400 Rs/ kg); Spinosad 45 SC 0.0101% (19000 Rs/ l); Thiodicarb 75 WP 0.0563% (3800 Rs/ kg); Novaluron 10 EC 0.0113% (3200 Rs/ kg); Labour charge (220 Rs); Price of okra fruit/ kg (12 Rs/ kg)

and Javed et al. (2018) on emamectin benzoate 5SG. Devi et al. (2015) and Yadav et al. (2017) revealed that emamectin benzoate 12 g a.i./ ha provided the best fruit protection against *E. vitella* on okra followed by spinosad 12.5% SC. Naveena et al. (2015) also obtained similar results with emamectin benzoate 5WG @ 7.50 g a.i/ha. Mane (2007), Dhar and Bhattacharya (2015) and Pachole et al. (2017) reported that spinosad at 45SC was the most effective against *E. vitella.* Effect of treatments on okra yield was found significant, and emamectin benzoate gave significantly higher yield (14444 kg/ha), at par with spinosad 45SC (14030 kg/ha). Economics of insecticides computed considering prevailing market price of okra and treatments including labour charges revealed that BCR was maximum of Rs. 126003 with ICBR of 1:2.66 in the treatment of emamectin benzoate followed by spinosad with Rs.116102, and ICBR of 1:2.22 (Table 1). These findings are in accordance with the results of Parmar and Borad (2009) on emamectin benzoate against *H. armigera.* Kuttalam et al. (2008) also reported similarly as that of Kumar et al. (2016) with spinosad. Pachole et al. (2017) revealed that spinosad 45SC was the best against *E. vitella* on okra and most economical. Results of pesticide residue analysis revealed that the most effective emamectin benzoate 5SG at 0.0025% showed the residues below quantification limit at five days after spray; and it was 0.223 ppm with spinosad 45SC at 0.0169%. These are below MRL.

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AUTHOR'S CONTRIBUTIONS

MMP: Performed field trials, analysed and interpreted the data of the work and prepared the original manuscript; RKS: Conducted field trials, analysed the data, reviewed and edited the writing, JRP: Assisted in conduction of field trials, analysed the data. MJJ: analysed the data, reviewed and edited the manuscripts. The authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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EFFICACY OF SOME INSECTICIDES AGANIST INVASIVE PIN WORM *TUTA ABSOLUTA* (MEYRICK) AND RED SPIDER MITE *TETRANYCHUS URTICAE* (KOCH) ON TOMATO

C M Karthik*, A P Biradar[1] and H T Prakash[1]

Department of Entomology, College of Agriculture, KSNUAHS, Shivamogga 577204, Karnataka, India
[1]Department of Entomology, College of Agriculture, Vijayapur, University of Agricultural Sciences, Dharwad 580005, Karnataka, India
*Email: karthikcmag62@gmail.com (corresponding author)

ABSTRACT

The pin worm *Tuta absoluta* (Meyrick) and red spider mite *Tetranychus urticae* (Koch) are major pests of tomato. A field experiment was carried out to evaluate the effectiveness of some insecticides against these at the College of Agriculture, Vijayapura, UAS, Dharwad. The results revealed that chlorantraniliprole 18.5%SC led to least number of larvae (1.60/ plant), live mines (1.26 live mines/ plant) and less fruit damage (2.06%) at fifth day of spray. Spiromesifen 22.9%SC led to significantly less number of mites (1.01/ square inch of leaf area) at third day of spray. The combination treatment of chlorantraniliprole 18.5%SC @ 0.15 ml/ l followed by spiromesifen 22.9%SC @ 0.5 ml/ l was found to be the best to manage both the pests.

Key words: Tomato, *Tuta absoluta, Tetranychus urticae,* chlorantraniliprole, spiromesifen, insecticides, live mines, fruit damage, combination

Tomato (*Lycopersicon esculentum*. L.) is an important vegetable crop mainly grown in tropics and subtropics, and falls under Solanaceae (Vavilov, 1951). Tomato pin worm *Tuta abosoluta* is an invasive alien pest species from South America which is spreading rapidly and causing considerable damage to tomato crop in recent years (Shashank et al., 2015). It is an oligophagous pest feeding on many related species of the family Solanaceae including tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.), pepper (*Capsicum annuum* L.), sweet pepino (*Solanum muricatum* L.), tobacco (*Nicotiana tabacum* L.), the jimson weed (*Datura stramonium* L.), the African eggplant (*Solanum aethiopicum* L.), and the European black nightshade (*Solanum nigrum* L.) (Desneux et al., 2018). Pin worm attacks the apical buds, flowers, and new fruits of tomato. Larvae make conspicuous mines and galleries on leaves and stems. Damage can occur at any stage of tomato growth from seedlings to mature plant. The larvae feed on the mesophyll tissue, leaving the epidermis intact, thus creating irregular mines and galleries on the leaves. The mines and galleries may become necrotic with time. These mining activities lead to reduction of the photosynthetic potential in the infested leaves (Biondi et al., 2018).

Mites of the family Tetranychidae are among the destructive pests of agricultural and horticultural crops. *Tetranychus urticae* Koch, the two-spotted mite is a polyphagous one and probably the most important agricultural mite pest (Khalighi et al*.,* 2016). It has reported on many plants like tomato, okra, brinjal, cotton, french bean, cucurbits, alfalfa, flowers, etc. (Manjulata et al., 2002). Under optimal conditions of high temperatures and low humidity, *T. urticae* can build to high densities and cause serious damage, especially in greenhouses. The mite generally feeds underneath the leaves and causes greying of the leaves due to mesophyll collapse followed by yellowing. It is estimated that 18-22 cells are destroyed per minute by a single mite. Continued feeding causes a stippled–bleached effect and later, the leaves turn yellow, grey or bronze. Complete defoliation may occur, if the mites are not controlled (Park and Lee, 2002). The present study evaluates combination spray of insecticides and acaricides at different intervals against *T. absoluta* and *T. urticae*.

MATERIALS AND METHODS

This study was conducted during kharif at the College of Agriculture, Vijayapura, University of Agricultural Sciences, Dharwad, during 2018. The experiment was conducted with eleven treatments and three replications, and the variety Lakshmi (hybrid) transplanted in July and grown following all recommended agronomic practices except for plant protection measures. The insecticides treatments were imposed two times as a spray in the cropping period at vegetative and fruit development stage after observing

pest incidence. The treatment details are: spiromesifen 22.9%SC @ 0.5ml/ l (T_1), dicofol 18.5%EC @ 2.5ml/ l (T_2), fenazaquin 10%EC @ 2.0 ml/ l (T_3), propargite 57%EC @ 3.0 ml/ l (T_4), chlorantraniliprole 18.5%SC @ 0.15 ml/ l (T_5), flubendiamide 39.35%SC @ 0.075 (T_6), emamectin benzoate 5%SG 0.20 g/ l (T_7), untreated check (T_8), chlorantraniliprole 18.5%SC @ 0.15 ml/ l followed by spiromesifen 22.9% SC @ 0.5 ml/ l (after one week spray of T_5) (T_9), chlorantraniliprole 18.5%SC @ 0.15 ml/ l followed by fenazaquin 10 % EC @ 2.0 ml/ l (after one week spray of T_5) (T_{10}) and chlorantraniliprole 18.5%SC @ 0.15 ml/ l followed by propargite 57%EC @ 3.0 ml/ l (after one week spray of T_5) (T_{11}). Five plants were randomly selected from each treatment and number of live mines and larvae/ plant was recorded at one day before spray and one, three, five, seven and 15 days after spray. Number of damaged fruits and healthy fruits were selected separately for calculating % fruit damage during harvesting. The % fruit damage by *T. abosoluta* was calculated by using the formula as described by Usman et al. (2012). Observation on the number of active mites/ square inch of leaf area (top, middle and bottom leaves of plant) was taken from five randomly selected plants of each treatment at one day before spray and one, three, five, seven and 15 days after spray.

RESULTS AND DISCUSSION

The number of *T absoluta* larvae/ plant varied from 1.60 to 8.43, with least value being with chlorantraniliprole 18.5%SC @ 0.15 ml/ l followed by propargite 57%C @ 3ml/ l(1.60/ plant); these were statistically on par with chlorantraniliprole 18.5%SC @ 0.15 ml/ l (1.63/ plant), as against untreated check showing maximum (9.61/plant) (Table 1). These results are in line with those of Sapkal et al. (2018) on chlorantraniliprole 18.5%SC, emamectin benzoate 5%SG, spinetoram 11.7%SC and spinosad 45%SC, of which chlorantraniliprole was the most effective. Kandil et al. (2020) observed that emamectin benzoate and chlorantraniliprole were the most superior. The least number of live mines were observed with chlorantraniliprole 18.5%SC @ 0.15 ml/ l (1.36/ plant), followed spiromesifen 22.9 % SC @ 0.5 ml/ l (1.41/ plant) (Table 1). Bassi et al. (2012) reported that chlorantraniliprole (Rynaxypyr) is a novel diamide insecticide with outstanding performance on *T. absoluta* in reduction of number of larvae and live mines. Dilipsundar and Srinivasan (2019) revealed maximum reduction in larval population with chlorantraniliprole 18.5 SC @ 40g a.i./ha (90.35%) followed by spinosad 45 SC @ 73g a.i./ha (87.58%) and flubendiamide 480 SC @ 48g a.i./ha (84.10%). The fruit damage was least in chlorantraniliprole 18.5%SC @ 0.15 ml/ l (2.06/ plant), followed by spiromesifen 22.9%SC @ 0.5 ml/ l (2.37/plant), while it was 24.99/ plant in untreated check (Table 1). Eleonora et al. (2014) reported that on the sixth day larval mortality was 100% for emamectin benzoate, flubendiamide and chlorantraniliprole. Ayalew (2015) revealed that fruit infestation in untreated control was between 54 and 76%, while in plots treated with diamide insecticides (chlorantraniliprole) it was significantly lower with 2–6% followed by spinosyns (spinetoram and spinosad) with 30–35% damage.

The incidence of mites/ square inch was the least in spiromesifen 22.9%SC @ 0.5 ml/ l (1.01/ sq inch) at third day and was on par with fenazaquin 10%EC @ 2ml/ l (1.14/ sq inch), while in untreated check it was 6.42/ vsq inch. Significantly on 15th day reduced mite incidence was observed with chlorantraniliprole 18.5%SC @ 0.15 ml/ l followed by spiromesifen 22.9%SC @ 0.5 ml/ l (1.63/ sq inch) (Table 1). Randhawa et al. (2020) showed that spiromesifen 22.9% @ 500 ml/ ha led to the least mite population (1.41mites/ leaf). Kavya et al. (2015) revealed that spiromesifen (1.05 mites/ leaf) reduced the incidence significantly than any other acaricides. Thus, chlorantraniliprole 18.5%SC @ 0.5ml/ l was superior against both *T. absoluta* and *T. urticae*, followed by flubendiamide 39.35%SC and emamectin benzoate 5% SG. The treatment spiromesifen 22.9% SC @ 0.5 ml/ l was effective in reducing *T. urticae* followed by fenazaquin 10%EC @ 2 ml/ l, propargite 57%EC @ 3ml/ l and dicofol 18.5%EC @ 2.5 ml/ l. The treatment chlorantraniliprole 18.5% SC @ 0.15 ml/ l followed by spiromesifen 22.9% SC @ 0.5 ml/ l was one of the best treatments as a combined approach.

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Table 1. Efficacy of insecticides against *T. absoluta* and *T. urticae* in tomato

Treatments	No. of *T. absoluta* larvae/ plant*						% fruit damage by *T. absoluta*/ plant+					
	1DBS	1DAS	3DAS	5DAS	7DAS	15DAS	1DBS	1DAS	3DAS	5DAS	7DAS	15DAS
T_1	7.80	7.87	7.97	8.12	8.23	8.43	21.87	22.23	22.49	23.26	24.50	25.23
	(2.88)	(2.89)^{a}	(2.91)^{b}	(2.94)^{b}	(2.96)^{b}	(2.99)^{b}	(27.87)	(28.12)^{ab}	(28.30)^{de}	(28.82)^{cd}	(29.66)^{c}	(30.14)^{c}
T_2	7.67	7.73	7.83	7.93	8.38	8.13	19.63	21.14	20.63	21.58	22.03	22.52
	(2.86)	(2.87)^{a}	(2.89)^{b}	(2.90)^{b}	(2.98)^{b}	(2.94)^{b}	(26.30)	(27.37)^{ab}	(27.01)^{d}	(27.68)^{c}	(27.99)^{b}	(28.33)^{b}
T_3	7.57	7.67	7.77	7.87	8.01	8.10	22.74	23.57	22.24	23.52	24.33	25.73
	(2.84)	(2.86)^{a}	(2.88)^{b}	(2.89)^{b}	(2.92)^{b}	(2.93)^{b}	(28.47)	(29.04)^{bc}	(28.14)^{de}	(29.01)^{cd}	(29.55)^{c}	(30.48)^{cd}
T_4	7.73	7.83	7.93	8.03	8.13	8.23	19.62	21.15	21.72	21.78	22.96	23.61
	(2.87)	(2.89)^{a}	(2.90)^{b}	(2.92)^{b}	(2.94)^{b}	(2.96)^{b}	(26.29)	(27.38)^{ab}	(28.16)^{de}	(28.38)^{cd}	(28.63)^{bc}	(29.07)^{bc}
T_5	7.93	7.10	2.80	1.63	2.30	3.60	21.01	19.84	9.15	2.06	4.41	5.90
	(2.90)	(2.76)^{a}	(1.82)^{a}	(1.46)^{a}	(1.67)^{a}	(2.02)^{a}	(27.27)	(26.45)^{a}	(17.59)^{a}	(8.54)^{a}	(12.12)^{a}	(16.14)^{a}
T_6	8.03	7.17	2.90	1.73	2.43	3.83	23.38	21.14	11.05	2.72	4.69	6.72
	(2.92)	(2.77)^{a}	(1.84)^{a}	(1.49)^{a}	(1.71)^{a}	(2.08)^{a}	(28.83)	(27.37)^{ab}	(19.41)^{bc}	(9.61)^{ab}	(12.50)^{a}	(15.02)^{a}
T_7	8.14	7.27	3.07	2.00	2.60	3.90	22.07	22.68	11.06	3.24	5.06	7.06
	(2.94)	(2.79)^{a}	(1.89)^{a}	(1.58)^{a}	(1.76)^{a}	(2.10)^{a}	(28.02)	(26.99)^{ab}	(20.79)^{c}	(10.13)^{b}	(13.25)^{a}	(15.39)^{a}
T_8	7.80	8.20	8.86	9.30	9.42	9.61	23.42	23.85	23.93	24.66	25.17	24.99
	(2.88)	(2.95)^{a}	(3.06)^{b}	(3.13)^{b}	(3.15)^{c}	(3.18)^{c}	(30.06)	(30.32)^{c}	(29.29)^{ef}	(29.66)^{de}	(30.11)^{cd}	(32.91)^{d}
T_9	7.70	7.13	3.00	1.80	2.20	3.57	24.22	22.39	10.81	2.37	4.45	6.61
	(2.86)	(2.76)^{a}	(1.87)^{a}	(1.52)^{a}	(1.64)^{a}	(2.02)^{a}	(29.48)	(26.95)^{a}	(19.52)^{bc}	(8.38)^{a}	(12.17)^{a}	(15.47)^{a}
T_{10}	7.93	7.17	2.90	1.73	2.30	3.70	25.04	20.02	10.51	2.60	4.53	6.65
	(2.90)	(2.77)^{a}	(1.84)^{a}	(1.49)^{a}	(1.67)^{a}	(2.05)^{a}	(29.11)	(26.58)^{a}	(18.92)^{ab}	(9.28)^{a}	(12.90)^{a}	(14.94)^{a}
T_{11}	7.73	7.00	2.96	1.60	2.20	3.77	22.35	20.06	10.14	2.61	4.52	6.69
	(2.87)	(2.74)^{a}	(1.86)^{a}	(1.45)^{a}	(1.64)^{a}	(2.07)^{a}	(28.21)	(26.60)^{a}	(18.56)^{ab}	(9.30)^{a}	(12.28)^{a}	(14.98)^{a}
S.Em.±		0.07	0.06	0.05	0.05	0.06		0.69	0.59	0.52	0.55	0.59
CD (p=0.05)	NS	0.21	0.17	0.16	0.15	0.17	NS	2.04	1.73	1.53	1.61	1.74
CV (%)		12.92	12.67	13.23	11.93	12.26		12.98	12.81	14.57	13.82	13.74

Treatments	No. of live mines of *T. absoluta* larvae/ plant						No. of mites/ square inch of leaf area					
	1DBS	1DAS	3DAS	5DAS	7DAS	15DAS	1DBS	1DAS	3DAS	5DAS	7DAS	15DAS
T_1	6.37	6.48	6.56	6.67	6.75	6.86	5.77	3.63	1.01	1.27	2.03	5.70
	(2.62)	(2.64)^{bc}	(2.66)^{bc}	(2.68)^{b}	(2.69)^{b}	(2.71)^{b}	(2.50)	(2.03)^{a}	(1.23)^{b}	(1.33)^{b}	(1.59)^{b}	(2.49)^{edcb}
T_2	6.79	6.30	6.41	6.50	6.60	6.69	5.40	4.65	1.40	1.57	2.46	5.53
	(2.70)	(2.61)^{bc}	(2.63)^{b}	(2.65)^{b}	(2.66)^{b}	(2.68)^{b}	(2.43)	(2.27)^{dcba}	(1.38)^{b}	(1.44)^{b}	(1.72)^{b}	(2.46)^{dcb}
T_3	6.23	6.33	6.43	6.52	6.63	6.73	4.99	4.05	1.14	1.32	2.12	5.09
	(2.59)	(2.61)^{bc}	(2.63)^{b}	(2.65)^{b}	(2.67)^{b}	(2.69)^{b}	(2.34)	(2.13)^{ba}	(1.28)^{b}	(1.35)^{b}	(1.62)^{b}	(2.36)^{b}
T_4	6.50	6.60	6.70	6.80	6.90	7.00	5.26	4.43	1.24	1.43	2.29	5.43
	(2.65)	(2.66)^{c}	(2.68)^{bc}	(2.70)^{b}	(2.72)^{b}	(2.74)^{b}	(2.40)	(2.22)^{cba}	(1.32)^{b}	(1.39)^{b}	(1.67)^{b}	(2.44)^{cb}
T_5	6.31	5.20	2.50	1.36	2.70	2.93	4.86	5.16	5.36	5.65	5.85	6.68
	(2.61)	(2.39)^{a}	(1.73)^{a}	(1.37)^{a}	(1.79)a	(1.85)^{a}	(2.32)	(2.38)^{dcb}	(2.42)^{dc}	(2.48)^{c}	(2.58)^{c}	(2.68)^{e}
T_6	6.47	5.30	2.60	1.46	2.70	2.83	5.27	5.55	5.85	6.05	6.21	6.31
	(2.64)	(2.41)^{a}	(1.76)^{a}	(1.40)^{a}	(1.79)^{a}	(1.83)^{a}	(2.40)	(2.46)^{dcb}	(2.52)^{dc}	(2.56)^{dc}	(2.59)^{dc}	(2.61)^{edc}
T_7	6.27	5.38	2.70	1.56	2.92	3.03	5.13	5.70	5.95	6.26	6.36	6.52
	(2.60)	(2.43)^{a}	(1.79)^{a}	(1.44)^{a}	(1.85)^{a}	(1.88)^{a}	(2.37)	(2.49)^{dc}	(2.54)^{dc}	(2.60)^{dc}	(2.62)^{dc}	(2.65)^{ed}
T_8	6.43	7.06	7.57	7.79	8.03	8.38	5.30	6.16	6.42	6.68	6.90	6.68
	(2.63)	(2.75)^{c}	(2.84)^{c}	(2.88)^{c}	(2.92)^{c}	(2.98)^{c}	(2.41)	(2.58)^{d}	(2.63)^{d}	(2.68)^{d}	(2.72)^{d}	(2.68)^{e}
T_9	6.37	5.24	2.55	1.41	2.75	2.80	4.93	5.85	6.05	6.16	6.52	1.63
	(2.62)	(2.40)^{a}	(1.75)^{a}	(1.38)^{a}	(1.80)^{a}	(1.82)^{a}	(2.33)	(2.52)^{dc}	(2.56)^{dc}	(2.58)^{dc}	(2.65)^{dc}	(1.46)^{a}
T_{10}	6.68	5.65	2.46	1.44	2.78	2.92	5.50	5.65	6.16	6.31	6.63	1.87
	(2.68)	(2.48)^{bc}	(1.72)^{a}	(1.39)^{a}	(1.81)^{a}	(1.85)^{a}	(2.45)	(2.48)^{dc}	(2.58)^{dc}	(2.61)^{dc}	(2.67)^{dc}	(1.54)^{a}
T_{11}	6.90	5.30	2.59	1.46	2.80	3.03	5.70	5.80	6.00	6.36	6.47	1.93
	(2.72)	(2.41)^{a}	(1.76)^{a}	(1.40)^{a}	(1.82)^{a}	(1.88)^{a}	(2.49)	(2.51)^{dc}	(2.55)^{dc}	(2.62)^{dc}	(2.64)^{dc}	(1.56)^{a}
SEm.±		0.05	0.06	0.05	0.06	0.06		0.10	0.05	0.05	0.05	0.06
CD (p=0.05)	NS	0.15	0.18	0.15	0.17	0.18	NS	0.28	0.15	0.14	0.15	0.17
CV (%)		10.58	13.93	13.86	13.64	14.23		11.97	12.63	12.52	12.79	13.94

DBS-Day before spray; DAS-Days after spary; *Figures in parentheses √(x+0.5) transformed; +Figures in parentheses arcsine transformed; Mean followed by similar alphabets in the column do not differ significantly at p=0.05% by DMRT

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EVALUATION OF COLLOIDAL CHITOSAN AGAINST DIAMOND BACK MOTH *PLUTELLA XYLOSTELLA* L. ON CAULIFLOWER

S Selva Rani, M Shanthi*, M Murugan[1], K Senthil[2], S Vellaikumar[3] and S Haripriya[4]

Department of Agricultural Entomology; [2] Department of Soils and Environment;
[3]Department of Biotechnology, Agricultural College and Research Institute,
Tamil Nadu Agricultural University (TNAU), Madurai 625104, Tamil Nadu, India
[1]Department of Agricultural Entomology; [4]Department of Nanoscience and Technology,
TNAU, Coimbatore 641003, Tamil Nadu, India
*Email: mshanthiento@tnau.ac.in (corresponding author)

ABSTRACT

An ecofriendly colloidal chitosan byproduct synthesized from crude chitosan was evaluated for its chronic toxicity against second instar larvae of *Plutella xylostella* L. by leaf dip bioassay. The consumption of 10000 ppm colloidal chitosan treated leaf caused 100% mortality after the 4th day, with least larval weight (0.02 mg). Whereas, the consumption of 8000 ppm treated leaves reduced the larval (0.65 mg), pupal (1.26 mg), and adult weights (0.75 mg), with 10% adult malformation and extended larval duration. This is compared to untreated larval duration of 10.66 days. Thus, colloidal chitosan exhibits chronic toxicity and growth inhibition effect on *P. xylostella* larvae.

Key words: *Plutella xylostella*, cauliflower, chitosan, synthesis, colloidal chitosan, glacial acetic acid, chronic toxicity, growth inhibition, malformation, larval period, larval weight

Diamond back moth (DBM) *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a widespread pest of cultivated and wild Brassicaceae viz., cabbage, cauliflower, broccoli, Brussel sprouts, radish, and field crops such as turnip, mustard, and rape. The pest can cause a high yield loss of 91.2% (Elizeu et al., 2020). Farmers apply insecticides at 6 to 10 days interval for 6 to 8 times to control this, and thus there is indiscriminate usage of insecticides. This results in residues, resurgence, resistance, and environmental hazards, warranting use of alternative ecofriendly insecticides. Recently, chitosan is gaining momentum in plant protection. Chitin is the most abundant biopolymer in marine environments (Souza et al., 2011), isolated from crustaceans such as crab and shrimp as well as from fungi (Kurita et al., 2000) and derived by deacetylation of chitin. It's a poly-b-(1™4)-D-glucosamine, and has attracted considerable attention for its potential applications in food, agriculture, medicine, pharmaceuticals, cosmetics, and wood preservation, due to its interesting physicochemical and biological properties (Eikenes et al., 2005; Torr et al., 2005; Du et al., 2009). It may also serve as a good alternative to pest control because of its insecticidal and antimicrobial properties (Rabea et al., 2003; Badawy et al., 2005). Its insecticidal effect against lepidopteran and homopteran insect pests is known, especially against *P. xylostella, Helicoverpa armigera, Spodoptera exigua,* and aphids *Hyalopterus pruni* (Zhang et al., 2003). Chitosan is an active insecticide against 4th instar larvae of *Spodoptera littoralis* (Sayed et al., 2014). The insect mortality can be achieved at its low dosage levels, and it is non-toxic to vertebrates and humans. Chitosan treatments are effective against herbivorous insect pests, but it has been used successfully as an ingredient in the artificial diet fed to carnivorous insects being reared for use in the biological control of chitinous pests (Tan et al., 2010). The present study focused on the synthesis of colloidal chitosan from chitosan and evaluation of its chronic toxicity and growth inhibition against diamond back moth *P. xylostella*.

MATERIALS AND METHODS

The synthesis and evaluation of colloidal chitosan were conducted in the Natural Pesticide Laboratory, Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai during 2020-2021. Colloidal chitosan was synthesized based on the method adopted by Cruz et al. (2004) with slight modification. Crude chitosan flakes were procured from MATSYAFED, Kerala State Co-operative Federation for Fisheries Development Ltd., Neendakara, Kerala. Crude chitosan flakes (10 g) were dissolved in 1500 ml of

0.2 N Hydrochloric acid for one hour by intermittent stirring and digested overnight. After digestion, pH was adjusted to 5.5 by using 0.2 N NaOH and 0.1 N HCl. After neutralization, the chitosan was centrifuged (model: Velocity 14R) at 7000 rpm for 5 min, the sediment was collected and freeze-dried in a lyophilizer (model: Scan Vac), and designated as colloidal chitosan.

Plutella xylostella culture was maintained by following the procedure described by Justin (1996). The effect of colloidal chitosan on the growth and development of *P. xylostella* was estimated by allowing the larvae to feed chronically on the colloidal chitosan-treated leaves (leaf dip bioassay) from second to final instar. The colloidal chitosan was dissolved in the solvent (1% glacial acetic acid), mixed with surfactant (Tween 80 0.05%), and prepared at different concentrations viz., 3000 ppm, 5000 ppm, 8000 ppm, 10000 ppm. Tween 80 0.05% and glacial acetic acid 1% were kept as negative checks, azadirachtin 1 EC @ 2 ml/ l was used as a treated check, in comparison with untreated check. The leaf bits (4x 4 cm) were prepared from young cauliflower leaves and used for the bioassay. Each treatment was replicated thrice, with each consisting of 10 second instar larvae. Larval weight was recorded daily. Pupal and adult weights were also noted. The % reduction in weight of larva, pupa, and adult over untreated check was estimated. If there were any malformations or mortalities during any life stages, these were also recorded. Larval, pupal period, adult life span, and the number of adults emerged were recorded. Adult emergence was estimated in % (Tian et al., 2020). All the experiments were conducted under a completely randomized block design (CRBD). Data were statistically analyzed using SPSS for Windows (version 22) (IBM Corp. Released, 2013) software to carry out ANOVA. Grouping of data was done by using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The mass of the colloidal chitosan (20 g) prepared from the raw material, called crude chitosan (10 g) was increased by two times. But, when the colloidal chitosan was freeze-dried, the mass was reduced to 5 g. It shows that the recovery yield of colloidal chitosan from crude chitosan was 50%. The raw chitosan was in the form of flakes and insoluble in water, hence it was transformed into colloidal form by deacetylation process, which had increased solubility in aqueous acidic solution, water binding capacity, and degree of deacetylation (%) (Selva Rani et al., 2021). The chronic feeding of *P. xylostella* on colloidal chitosan-treated leaves, at all concentrations, resulted in an efficient reduction in the growth and development of larva compared to the untreated check. The weight of the larva fed on colloidal chitosan 10000 ppm and azadirachtin 1 EC @ 2 ml lit^{-1} was very minimum after the third day of feeding (D7) (0.10 mg and 0.06 mg, respectively). The next effective treatment was colloidal chitosan 8000 ppm (0.37 mg), while the untreated check had 1.19 mg on the same day. The maximum larval weight attained in 8000 ppm and the untreated check was 0.92 mg and 5.97 mg per larva, respectively. Subsequently, there was a significant reduction in pupal and adult weight also (Table 1).

This significant reduction in larval, pupal, and adult weight after feeding on colloidal chitosan 8000 ppm was noticed (91.57%, 74.38% and 66.32%, respectively). Consequently, the larval period of *P. xylostella* was prolonged by two days (12.66 days) compared to untreated check (10.66 days). In the case of pupal period and adult life span, no significant difference was found among the treatments. From the chronic feeding study, it was found that 10000 ppm of colloidal chitosan and azadirachtin 1 EC caused 100% larval mortality on the fourth day after treatment, hence no adults were emerged in these treatments. While, 8000 ppm colloidal chitosan caused larval mortality to an extent of 63.33% and malformations of larva, pupa and adult to a level of 23.33, 3.33, and 10.0%, respectively and no normal adults were emerged (Table 1). These results revealed the efficacy of the colloidal chitosan at 10000 and 8000 ppm in causing chronic toxicity and growth inhibition in *P. xylostella*. At the same time, there was no mortality and growth inhibition of larva, pupa, and adult in the untreated check.

Chitosan caused 72% of mortality of *P. xylostella* due to the formation of the film on the surface of insects, which block the air while breathing that resulting in asphyxiation and ultimate death (Zhang et al., 2003). In another study, chitosan derivative (*N*- (2- chloro- 6- fluorobenzyl) was found effective against *S. littoralis* with an LC50 of 0.32 g kg^{-1} diet and 100% mortality at ≥0.625 g kg^{-1} (Badawy et al., 2012). Typically, colloidal chitosan inhibited the larval growth in a time-dependent manner from the first day of feeding on the treated leaf. Unmodified chitosan caused 62.72% mortality and also 27% reduction of *S.litura* larval growth after 7 days of treatment (Uddin et al., 2021). An early study of *N*-alkyl chitosan (NAC) derivatives against *S.litura* reported that insect growth was significantly decreased and the

Table 1. Effect of chronic feeding of colloidal chitosan on *P. xylostella.*

Treatments	Reduction in weight over untreated (%)*$			Developmental period (days)*#			Larval morality (%)*$		Pupal morality (%)*$		Adult emergence (%)*$	
	Larval weight	Pupal weight@	Adult weight@	Mean Larval Period+	Mean pupal period+	Mean adult life span+	Normal@	Malformed@	Normal@	Malformed@	Normal@	Malformed@
T1– Colloidal chitosan - 3000 ppm	75.82± 0.74 (60.52)^{d}	47.07± 5.38 (43.32)^{c}	39.64± 2.75 (39.02)^{c}	11.00± 0.00 (3.31)^{b}	5.00± 0.00 (2.23)	14.66± 0.57 (3.82)	33.33± 0.00 (35.26)^{d}	6.66± 0.57 (14.96)^{b}	-	0.00± 0.00 (0.91)^{b}	53.33± 0.57 (54.73)^{b}	6.66± 0.57 (14.96)^{a}
T2 – Colloidal chitosan -5000 ppm	81.98± 3.06 (64.85)^{c}	58.60± 1.61 (49.95)^{b}	53.37± 1.34 (46.93)^{b}	11.00± 0.00 (3.31)^{b}	5.33± 0.57 (2.30)	14.00± 0.00 (3.74)	43.33± 0.57 (41.16)^{c}	6.66± 0.57 (14.96)^{b}	-	3.33± 0.57 (10.51)^{a}	40.00± 0.57 (39.23)^{c}	6.66± 0.57 (14.96)^{a}
T3 – Colloidal chitosan - 8000 ppm	91.57± 1.87 (73.09)^{b}	74.38± 0.82 (59.59)^{a}	66.32± 4.46 (54.53)^{a}	12.66± 0.57 (3.55)^{a}	5.33± 0.57 (2.30)	0.00± 0.00 (0.91)	63.33± 0.57 (52.73)^{b}	23.33± 0.57 (28.88)^{a}	-	3.33± 0.57 (10.51)^{a}	0.00± 0.00 (0.91)^{d}	10.00± 0.00 (18.43)^{a}
T4 – Colloidal chitosan - 10000 ppm	99.63± 0.43 (86.49)^{a}	0.00± 0.00 (0.91)^{e}	0.00± 0.00 (0.91)^{e}	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)	0.00± 0.00 (0.91)	100.00± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	0.00± 0.00 0.91)^{d}	0.00± 0.00 (0.91)^{b}
T5 – Acetic acid 1%	2.88± 0.44 (9.76)^{e}	2.46± 3.03 (9.02)^{d}	1.55± 0.61 (7.16)^{d}	10.33± 0.57 (3.21)^{b}	5.00± 0.00 (2.23)	14.33± 0.57 (3.78)	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	100.00± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{b}
T6 –Tween80 0.05%	4.67± 2.02 (12.48)^{e}	1.69± 1.17 (7.46)^{d}	0.62± 0.31 (4.52)^{d}	10.66± 0.57 (3.26)^{b}	5.00± 0.00 (2.23)	14.66± 0.57 (3.89)	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	100.00± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{b}
T7 – Azadirachtin 1 EC @ 2 ml/ l	99.24± 0.86 (84.97)^{a}	0.00± 0.00 (0.91)^{e}	0.00± 0.00 (0.91)^{e}	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)	0.00± 0.00 (0.91)	100.00± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	0.00± 0.00 (0.91)^{d}	0.00± 0.00 (0.91)^{b}
T8 – Untreated check	-	-	-	10.66± 0.57 (3.26)^{b}	5.00± 0.00 (2.23)	14.33± 0.57 (3.78)	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	100± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{b}
SEd	1.23*	1.86*	1.76*	0.33*	NS(0.24)	NS(0.43)	0.28	0.43	-	0.27	0.16	0.27

Treatments	Mean fresh weight of larvae (mg)+						Cumulative daily larval mortality (%)@			
	Initial weight	3 DAT	4 DAT	5 DAT	6DAT	7DAT	1DAT	2DAT	3DAT	4DAT
T1– Colloidal chitosan - 3000 ppm	0.11± 0.01 (0.33)^{a}	0.79± 0.02 (0.89)^{c}	1.13± 0.04 (1.06)^{b}	1.36± 0.11 (1.17)^{c}	1.42± 0.09 (1.19)^{b}	1.52± 0.04 (1.23)^{ab}	0.00± 0.00 (4.05)^{d}	13.33± 0.57 (21.40)^{d}	23.33± 0.47 (28.88)^{c}	23.33± 0.57 (28.88)^{d}
T2 – Colloidal chitosan -5000 ppm	0.11± 0.02 (0.33)a	0.72± 0.09 (0.85)^{c}	1.10± 0.02 (1.05)^{b}	0.71± 0.18 (0.84)^{b}	1.05± 0.05 (1.02)^{a}	1.16± 0.17 (1.23)^{ab}	3.33± 0.47 (10.51)^{d}	20.00± 0.00 (26.55)^{d}	30.00± 0.81 (33.19)^{c}	36.66± 0.57 (37.26)^{c}
T3 – Colloidal chitosan - 8000 ppm	0.11± 0.01 (0.33)^{a}	0.37± 0.09 (0.61)^{b}	0.94± 0.10 (0.97)^{a}	0.49± 0.18 (0.70)^{a}	0.92± 0.25 (0.96)^{a}	0.65± 0.13 (0.81)^{a}	13.33± 0.47 (21.40)^{c}	43.33± 0.57 (41.15)^{c}	50.00± 0.00 (50.74)^{b}	53.33± 0.57 (46.91)^{b}
T4 – Colloidal chitosan - 10000 ppm	0.10± 0.01 (0.32)^{a}	0.10± 0.04 (0.32)^{a}	Dead	Dead	Dead	Dead	26.66± 0.47 (31.07)^{b}	66.66± 0.57 (54.71)^{b}	83.33± 0.47 (65.87)^{a}	100.00± 0.00 (90.00)^{a}
T5 – Acetic acid 1%	0.10± 0.01 (0.32)^{a}	1.18± 0.01 (1.08)^{d}	2.03± 0.01 (1.43)^{c}	4.05± 0.06 (2.01)^{d}	5.58± 0.10 (2.36)c	5.28± 1.08 (2.30)^{bc}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (0.66)^{e}
T6 –Tween80 0.05%	0.10± 0.01 (0.32)^{a}	1.18± 0.03 (1.08)^{d}	2.07± 0.07 (1.44)^{c}	4.06± 0.04 (2.01)^{d}	5.69± 0.11 (2.38)^{c}	3.63± 3.16 (1.90)^{b}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}
T7 – Azadirachtin 1 EC @ 2 ml/l	0.10± 0.00 (0.32)^{a}	0.06± 0.06 (0.25)^{a}	Dead	Dead	Dead	Dead	40.00± 0.81 (39.23)^{a}	76.66± 1.15 (61.09)^{a}	90.00± 0.00 (71.53)^{a}	100.00± 0.00 (90.00)^{a}
T8 – Untreated check	0.11± 0.01 (0.33)^{a}	1.19± 0.02 (1.09)^{d}	2.08± 0.03 (1.44)^{c}	4.27± 0.05 (2.07)^{e}	5.66± 0.14 (2.87)^{c}	5.97± 0.06 (2.44)^{c}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}
SEd	0.006	0.044	0.038	0.087	0.106	0.97	0.40	0.37	0.36	0.30

*Mean values of three replications as $\bar{x}$± SD; +,#Figures in parentheses square root transformed values +(√x+0.5); @,$Figures in parentheses arc sine transformed values @ (x+0.5); mean followed by same letter not significantly different from each other, DMRT ($p \leq 0.05$); SEd: Standard error of difference.

normal ecdysis process was affected, with symptoms of inhibition of feeding and weight gain, and the larvae were very small compared with the controls (Rabea et al., 2006). Colloidal chitosan 7% caused 85.38 % antifeedant activity against the first instar larva of *S. frugiperda,* further it inhibited the larval growth in a time-dependent manner from the first day of feeding (Moorthy et al*.,* 2021). Chitosan mixture (chitosan with secondary metabolites of *B. bassiana*) showed growth inhibition of 80.68% at 3000 ppm concentration against third instar larva of *S. littoralis* (Abdullah and Sucker, 2021). Thus, the present study observed that colloidal chitosan possesses the toxic and growth inhibition effect on *P. xylostella* larva upon chronic feeding. Hence, it could be explored further as an ecofriendly biomolecule.

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NATURAL ENEMIES ASSOCIATED WITH SORGHUM SHOOT BUG *PEREGRINUS MAIDIS* (ASHMEAD)

Saicharan Dharavath*, S S Karabhantanal and S B Jagginavar

Department of Agricultural Entomology, University of Agricultural Sciences Dharwad, College of Agriculture, Vijayapur 586101, Karnataka, India
*Email: charan.dharavath@gmail.com (corresponding author)

ABSTRACT

This study on the potential natural enemies associated with sorghum shoot bug *Peregrinus maidis* (Ashmead) revealed that among the predators, the predatory bug [*Creontiades* sp. and *Tytthus parviceps* (Reuter)] and neuropterans [*Chrysoperla* sp. and *Micromus timidus* (Hagen)] were found predating over eggs and nymphs. Beetles of Coccinellidae [*Cheilomenes sexmaculata* (Fab.)] and Chrysomelidae [*Monolepta signata* (Olivier)] were also found predating but only on nymphs. Six spiders were observed predating over nymphs and adults- these include *Callitrichia* sp. (Linyphiidae), *Cheiracanthium approximatum* (Cheiracanthiidae), *Marengo* sp. (Salticidae), *Neoscona* sp. (Araneidae), *Plexippus petersi* (Salticidae) and an unidentified Linyphiidae. A parasitoid *Anagrus* sp. (Hymenoptera: Mymaridae) was on the eggs and a mite *Erythraeus* sp. (Trambidiformes: Erythridae) was noticed adhering to the adults. This study is the first record of *Creontiades* sp.

Key words: *Peregrinus maidis*, sorghum, *Cheilomenes sexmaculata*, *Creontiades*, *Erythraeus*, *Marengo*, *Monolepta signata*, *Neoscona*, parasitoid, *Tytthus parviceps*

Shoot bug *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) is a major sucking pest of sorghum in the northern dry zone of Karnataka, given only minor importance earlier, but now it is a major pest status in rabi sorghum causing direct and indirect loss. The nymphs and macropterous females are more efficient transmitters of maize stripe virus of sorghum (MStV-S), maize mosaic virus of sorghum (MMV-S) and sorghum stripe disease virus (SStdv) compared to its males. The occurrence of MStV-S was first reported in India during the 1990s (Peterschmitt et al., 1991). The use of insecticides to tackle *P. maidis* in rabi sorghum by small and marginal farmers under rainfed situations is not a reasonable option because of its prohibitive cost and low returns in addition to toxicity hazards to the environment (Sharma et al., 2003). Unlike brown plant hopper *Nilaparvatha lugens* (Stal.), the natural enemies associated with *P. maidis* under field situation and their role in supressing shoot bug population was not fully understood although they belong to the same family. Further, the reports relating to natural enemies are scanty and need to be focused. The present study identifies the natural enemies associated with *P. maidis* to enable biological control.

MATERIALS AND METHODS

This study was done at Vijayapura in the Northern Dry Zone (Region-II, Zone-3) of Karnataka (16º 49'N, 77º20'E, 398.37 masl) during rabi 2020-21 on the Hathi Kunta, a susceptible sorghum variety. The crop was sown under unprotected conditions in 20 m^2 plots in three locations at the Regional Agricultural Research Station (RARS), Vijayapur. The parasitoids and predators observed associated with *P. maidis* at different intervals were recorded, collected, and preserved. These were evaluated for predation under laboratory conditions. The collected specimens were sent to the Department of Agricultural Entomology, University of Agricultural Sciences, GKVK, Bangalore and NBAIR, Bangalore for identification.

RESULTS AND DISCUSSION

Results of the present study on the natural enemies of *P. maidis* revealed a parasitoid fairy fly, *Anagrus* sp. (Hymenoptera: Mymaridae) collected from the parasitized eggs (Table 1). The adult was very small, 1 mm in width (Fig. 1a), like a miniature ant with reddish brown coloured body and hardly noticeable with naked eyes. *Anagrus* sp. parasitizes the eggs which were already inserted inside the midribs of sorghum leaves by inserting its ovipositor (Fig. 1b). The adult parasitoid parasitized one egg/ min. Similar observations were made by Guppy (1914) who reported hymenopteran parasite *Anagyrus flaveolus* (Watern) parasitising eggs of *P. maidis* to the extent of 75 to 80%. Muir (1917) and Perkins (1905) also reported that the eggs were

parasitised by *Paranagrus* sp. The predators collected from the sorghum ecosystem revealed that predatory bug, *Creontiades* sp. (Fig. 2) predates on the eggs and nymphs in the sorghum whorls. Several mirid bugs had been earlier reported as egg predators but this study observed *Creontiades* sp. as both egg and nymphal predator. The predation was high in case of egg stage with ten eggs/ min whereas, it fed only single nymph for more than a minute. The adult was 13 to 15 mm long and 3-5 mm in width, with abdomen being 7-9 mm long; adult is slender, pale green with translucent wings; antennae longer than the body and similarly, the wings also ascend the abdomen which bears membranous part apically black; abdomen is telescopic in which the segmentation was clearly distinct. Another predatory bug *Tytthus parviceps* (Reuter) (Hemiptera: Miridae) (Fig. 3) was found predating on both eggs and nymphal stages during the vegetative and flowering stages; the former is larger than the latter with more predation being from *Creontiades* sp. Neuropteran predators viz., *Chrysoperla* sp. (Chrysopidae) and *Micromus timidus* (Hagen) (Hemerobiidae) predate on nymphs. Similar observations on two mirid bugs i.e., *Tytthus mandulus* Bredd. and *T. parviceps* (Reut.) predating over the eggs of *P. maidis* was made in sorghum- Swezey (1936), Carnegie and Harris (1969), and Napompeth (1973); Swezey (1936) reported *Chrysoperla basalis* Walker (Neuroptera: Chrysopidae) predating on both nymphs and adults. Rioja et al. (2006) observed *Chrysoperla* sp.and *Chrysoperla 7-punctata* var. brucki on nymphs.

The Coccinellidae predators such as *Cheilomenes sexmaculata* (F) and the Chrysomelidae *Monolepta signata* (Olivier) were observed on the nymphs, found all the three crop growth phases; the coccinellids were highly polyphagous over soft bodied insects, and were found feeding on aphids; their grubs are very active and predated all body parts including head; but the chrysomelid was found feeding only on the soft body parts i.e., the abdomen of nymphs. These results corroborate with those of Singh et al. (1993) on *C. sexmaculata*. Predatory mite *Erythraeus* sp. (Trambidiformes: Erythridae) (Fig. 4b) was noticed on the adults and found adhering or clinging on the thoracic region (Fig. 4a). Kulkarni et al. (1979) from Dharwad identified the predacious mite *Erythraeus* sp. feeding on *P. maidis*. Some spiders found feeding on the nymphal and adult stages include- *Callitrichia* sp. (Araneae: Linyphiidae) (Fig. 5), *Cheiracanthium approximatum* (Araneae: Cheiracanthiidae) (Fig. 6), *Marengo sp.* (Araneae: Salticidae) (Fig. 7), *Neoscona* sp. (Araneae: Araneidae) (Fig. 8) and *Plexippus petersi* (Araneae: Salticidae) (Fig. 9)- these were found predating on nymph and adult stages. Of these *Callitrichia* sp. was smaller, and found abundantly with high predation rate. Similar findings were also made by Napompeth (1973) who reported *Hasarius adansoni* (Aud.) of Araneidae predating on both adult and nymph in Hawaii. This study further hinted a rich community of spiders belonging to the families Lycosidae, Linyl phiidae and Tetragnathidaea as potential predators of nymphs and adults.

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Table 1. Parasitoids and predators observed on *P. maidis*

Species identified	Order	Family	Stage of predation	No. of eggs/ nymphs predated/ min
Anagrus sp.	Hymenoptera	Mymaridae	Egg (parasitisation)	1 egg
Cheilomenes sexmaculata (Fabricius)	Coleoptera	Coccinellidae	Nymphs	1 nymph
Creontiades sp.	Hemiptera	Miridae	Egg & nymphs	10 eggs & 1 nymph
Tytthus parviceps (Reuter)	Hemiptera	Miridae	Egg & nymphs	5 eggs & 1 nymph
Chrysoperla sp.	Neuroptera	Chrysopidae	Nymphs	1 nymph
Micromustimidus Hagen	Neuroptera	Hemerobiidae	Nymphs	1 nymph
Monolepta signata (Olivier)	Coleoptera	Chrysomelidae	Nymphs	1 nymph
Erythraeus sp.	Trombidiformes	Erythridae	Adults	----
Callitrichia sp.	Araneae	Linyphiidae	Nymphs & adults	1 nymph/ adult
Cheiracanthium approximatum	Araneae	Cheiracanthiidae	Nymphs & adults	1 nymph/ adult
Marengo sp.	Araneae	Salticidae	Nymphs & adults	1 nymph/ adult
Neoscona sp.	Araneae	Araneidae	Nymphs & adults	1 nymph/ adult
Plexippus petersi	Araneae	Salticidae	Nymphs & adults	1 nymph/ adult
Unidentified	Araneae	Linyphiidae	Nymphs & adults	1 nymph/ adult

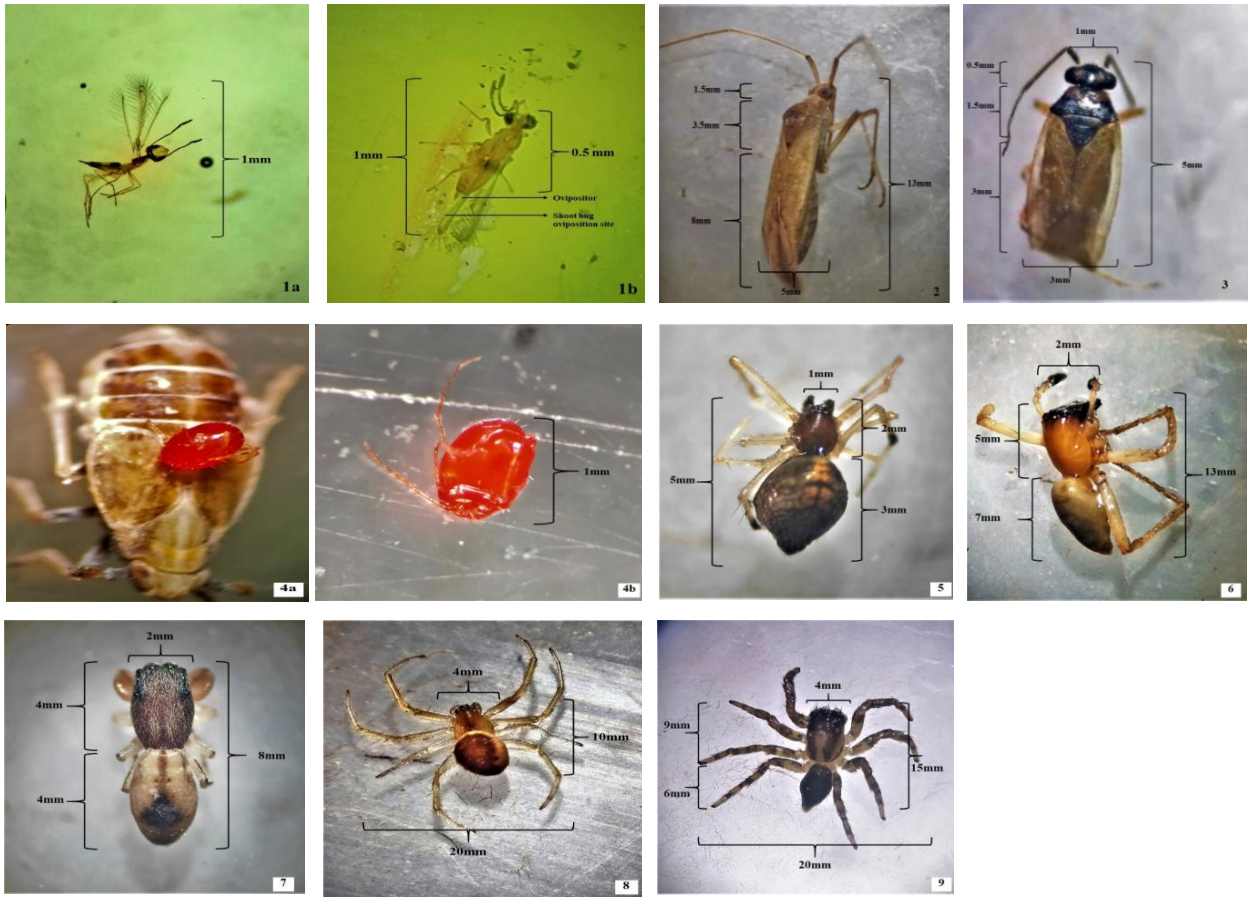

Fig. 1a. *Anagyrus* sp. 1b. *Anagyrus* sp. parasitizing shoot bug egg; 2. *Creontiades* sp.; 3. *Tytthus parviceps*; 4a. *Erythraeus* sp. clinging to thoracic region of shoot bug; 4b. *Erythraeus* sp.; 5. *Callitrichia* sp.; 6. *Cheiracantheum approximatum*; 7. *Marengo* sp.; 8. *Neoscona* sp.; 9. *Plexippus petersi*

Agricultural Sciences, GKVK, Bangalore; Dr Sampath Kumar M, Scientist (Agricultutural Entomology), ICAR-NBAIR Bengaluru, and Biswamitra Reang, PG Student, IGKV, Raipur for identification of samples.

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TOXICITY OF *TRIDAX PROCUMBENS* LEAF EXTRACT TO DENGUE VECTORS *AEDES AEGYPTI* L. AND *AE ALBOPICTUS* SKUSE

Grace Marin, Subramanian Arivoli[1] and Samuel Tennyson[2]*

Department of Zoology, Scott Christian College, Nagercoil 629003, Tamil Nadu, India
[1]Department of Zoology, Thiruvalluvar University, Vellore 632115, Tamil Nadu, India
[2]Department of Zoology, Madras Christian College, Chennai 600059, Tamil Nadu, India
*Email: samtennyson@gmail.com (corresponding author)

ABSTRACT

Insecticides of plant origin are effective in mosquito control owing to its mode of action. In the present study, *Tridax procumbens* L. ethanolic leaf extract was found to be toxic to the larvae of *Aedes aegypti* L. and *Ae. albopictus* Skuse as these caused 100% mortality in *Ae. aegypti* and 97% mortality in *Ae. albopictus* after 72 hr, with LC_{50} values of 288.40, 120.33 and 77.62 mg/ l for *Ae. aegypti*, and 812.83, 338.84 and 128.83 mg/ l for *Ae. albopictus* after 24, 48 and 72 hr, respectively. The toxicity of *T. procumbens* ethanolic leaf extract on larvae of *Aedes* spp., could be attributed to the presence of phytochemical compounds, viz., betulin, betulinic acid, lupeol (triterpenoid), caryophyllene, isophytol, phytol (terpene), limonene (monoterpene), luteolin (flavonoid), stigmast-5-en-3-ol, 3 (Beta)-, stigmast-5-en-3-ol, oleate, and palmitic acid (sterol) revealed by the gas chromatography-mass spectrometry analysis.

Key words: *T. procumbens*, leaf, ethanol, Gas chromatography-mass spectrometry (GC-MS), phytochemical constituents, *Ae. aegypti*, *Ae. albopictus*, larvicidal, toxicity

Man suffers extensively due to the nuisance of insect, particularly mosquitoes in health point of view as they directly transmit diseases (WHO, 2017; Chala and Hamde, 2021). *Aedes* is a genus of mosquitoes, originally found in tropical zones of Southeast Asia including India. *Aedes aegypti* and *Ae. albopictus* are responsible for the transmission of dengue fever. According to WHO (2021), the diseases transmitted by *Aedes* spp., are serious in the field of public health. The principal method by which mosquito/ vector-borne diseases are controlled is through vector control, which has a long and distinguished history (Wilson et al., 2020). There is a need to return to vector control approaches, which utilize a range of insecticides. Synthetic chemicals are effective, nonetheless, cause adverse effects on the environment and human health (van den Berg et al., 2021). Due to their hazardous side effects, ecofriendly alternatives are required for safer mosquito management. One such alternative approach is to explore the floral biodiversity and use these as insecticides of botanical origin (Nathan, 2020). Such a search for natural mosquitocides is ongoing, as the phytochemicals from plant origin have multiple modes of action (Smith et al., 2021). The development of botanical insecticides have become more rigorous in recent years with calls for more standardization, especially against mosquito larvae (Shaalan et al., 2005; Sakthivadivel and Daniel, 2008; Samuel et al., 2012a, b; Arivoli et al., 2012a, b; Ghosh et al., 2012; Kishore et al., 2014; Samuel and William, 2014; Raveen et al., 2017; Pavela et al., 2019; Nathan, 2020). In addition to the direct use of phytoextracts, biosynthesized phytonanopesticides are also gaining momentum as biocontrol agents against mosquitoes (Samuel et al., 2016). *Tridax procumbens* extracts have proved to be effective against mosquito larvae, however few reports present the larvicidal activity of its ethanolic extract (Macedo et al., 1997; Elumalai et al., 2013). Therefore, *T. procumbens* ethanolic leaf extract's phytochemical profile (GC-MS) analysis, and its toxicity against *Ae. aegypti* and *Ae. albopictus* larvae have been assessed in this study.

MATERIALS AND METHODS

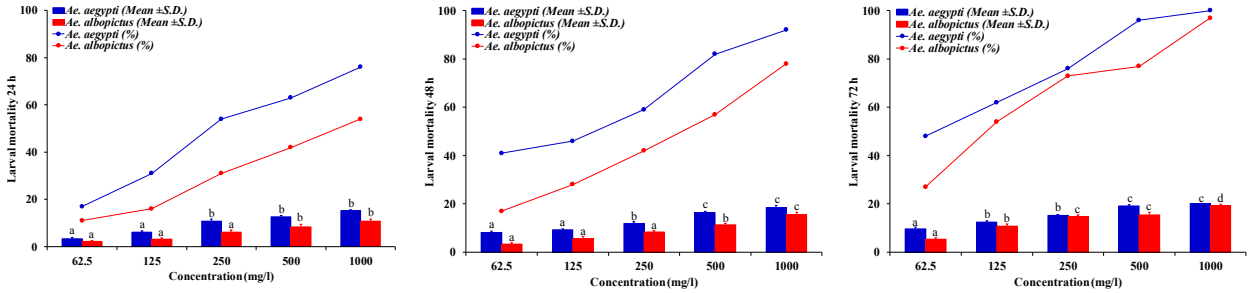
Mature, fresh and healthy leaves of *T. procumbens* collected from Nagercoil, Kanyakumari, Tamil Nadu, India were brought to the laboratory, and taxonomically identified at the Department of Botany, Scott Christian College, Nagercoil, Kanyakumari, Tamil Nadu, India. The collected leaves were washed with dechlorinated water, and shade dried at room temperature. Thereafter, the dried leaves were coarsely powdered by an electric blender, and sieved by a kitchen sieve. Finely powdered leaf (1 kg) was extracted with ethanol (3 l) in a Soxhlet

apparatus. The crude ethanolic leaf extract thus obtained was stored in air tight amber-coloured bottles at 4°C for bioassay. Clarus 680 GC was used for GC-MS analysis of *T. procumbens* ethanolic leaf extract to obtain its phytochemical profile in the Sophisticated Instrumentation Facility, Chemistry Division, School of Advanced Science, VIT University, Vellore, Tamil Nadu, India. *Aedes* immatures collected from Nagercoil, Kanyakumari, Tamil Nadu, India with an aid of a dipper were transported in plastic containers to laboratory, and thereafter moved to enamel larval salvers till adult emergence. Adults were identified and species confirmed prior to rearing (Tyagi et al., 2015). Subsequently, their cyclical generations were provided a blood meal, and was maintained in two feet mosquito cages (27 ±2°C, 70-80% RH) inside an insectary. Ovitraps inside the mosquito cages collected the oviposited eggs which were shifted to the larval rearing room in enamel larval salvers, and the larvae on hatching were provided larval food (yeast and dog biscuits of ratio 1:3). The larvae on turning into pupae were moved to another mosquito cage in enamel bowls for adult emergence.

World Health Organization (WHO) protocol was adopted for the study with minor modifications (WHO, 2005). Serial dilution of 1.0% stock solution of the crude phytoextract yielded requisite test concentrations (62.5, 125, 250, 500, and 1000 mg/ l) and amount of test solution. Early third instar larvae obtained from laboratory colonized F_1 generation was tested. The early third instars numbering 20 were added into glass beakers (250 ml) holding distilled water and test concentration for each replicate apiece trial. Distilled water (250 ml), and Tween 80 (1.0 ml) dissolved in distilled water (249 ml) maintained separately and run simultaneously served as positive and negative control, respectively. Larval mortality was confirmed when the moribund larvae showed no signs of movement when prodded by a needle on their respiratory siphon, and were scored dead. Mortality was observed 24, 48 and 72 hr after treatment. A total of five replicates, a negative and positive control were run concurrently for every trial, and overall five trials were run. Larval mortality in % was calculated, and when control mortality ranged from 5-20%, it was corrected by Abbott's (1925) formula. All mortality data were subjected to probit analysis, chi-square and regression analysis. One-way analysis of variance with Tukey's honestly significant tests was done to differentiate mean mortality. The differences were considered as significant at $p \leq 0.05$ level. Statistical analyses were carried in IBM SPSS Statistics v22 (SPSS, 2010).

RESULTS AND DISCUSSION

The phytochemical profile of *T. procumbens* ethanol leaf extract by GC-MS analysis revealed the presence of flavonoids, phenols, saponins, steroids, sterols, tannins, terpenes and terpenoids, and its phytochemical compounds were betulin, betulinic acid, caryophyllene, hydroquinone, isophytol, limonene, linoleic acid, lupeol, luteolin, myristic acid, 4-octanol, oleic acid, palmitic acid, pentadecanoic acid, phytol, salicylic acid, stearic acid, stigmast-5-en-3-ol, 3 (Beta)-, stigmast-5-en-3-ol oleate, squalene, tridecylic acid, and undecylic acid. The mortality of *Ae. aegypti* and *Ae. albopictus* larva exposed to various concentrations of *T. procumbens* ethanolic leaf extract after 24, 48 and 72 hr are presented in Fig. 1. No larval mortality was observed in both controls. Complete mortality was observed in *Ae. aegypti* larvae followed by 97% in *Ae. albopictus* at the highest concentration after 72 hr of exposure. *T. procumbens* extracts gave LC_{50} values of 288.40, 120.33 and 77.62 mg/ l for *Ae. aegypti* and 812.83, 338.84 and 128.83 mg/ l for *Ae. albopictus* after 24, 48 and 72 hr, respectively. One way ANOVA, comparing treated and control group ($p<0.05$) showed that *T. procumbens* concentrations significantly influenced the mortality of *Aedes* larvae (Table 1). Overall results



Different superscript alphabets indicate statistical significant difference in larval mortality between concentrations at $p<0.05$ level, one way ANOVA followed by Tukey's test

Fig. 1. Larval mortality of *Aedes* spp. on exposure to *T. procumbens* ethanolic leaf extract

Table 1. Probit analysis and other associated statistical inferences for the present study

Hours	LC_{50} (mg/l)	LC_{90} (mg/l)	Chi-square	Regression equation	R^2	F	P
			Ae. aegypti				
24	288.40	2454.70	0.418	Y=0.011X+5.300	0.7943	4.912	<0.057
48	120.23	954.99	1.234	Y=0.011X+8.525	0.8905	4.830	<0.059
72	77.62	380.19	1.596	Y=0.010X+11.317	0.7775	4.770	<0.600
			Ae. albopictus				
24	812.83	4069.78	0.135	Y=0.008X+2.725	0.895	5.003	<0.055
48	338.84	2754.23	0.214	Y=0.012X+4.141	0.939	4.932	<0.057
72	128.83	616.60	1.642	Y=0.012X+8.441	0.757	4.822	<0.059

indicated that the *T. procumbens* ethanolic leaf extracts are more toxic on *Ae. aegypti* than *Ae. albopictus*.

The results of the present study were comparable with the earlier reports of *T. procumbens* ethanolic extracts against mosquito larvae. Macedo et al. (1997) screened ethanolic aerial extracts of 83 plants belonging to Asteraceae family for larvicidal activity against *Aedes fluviatilis* Lutz of which 27 caused significant lethality. Elumalai et al. (2013) reported its aqueous, chloroform, ethanol, petroleum ether and methanolic leaf extracts to possess larvicidal activity against *Ae. aegypti* (LC_{50} 83.40, 108.22, 55.67, 94.13 and 56.02 ppm), *An. stephensi* (LC_{50} 92.79, 104.73, 77.70, 117.09 and 66.66 ppm) and *Cx. quinquefasciatus* (LC_{50} 80.58, 97.93, 57.46, 111.48 and 60.31 ppm). The susceptibility of larvae to botanical insecticides depends in general on the solvent extract, and the mosquito species tested. In order to get a potent extract, prior to selection of solvents, a thorough knowledge on the phytochemical profile of the plant/plant part used should be drawn, as there exists a relationship between the extract effectiveness and solvent polarity. The choice of solvent is influenced by what is intended with the extract, as it targets the compounds to be extracted (Ghosh et al., 2012). In the present study, ethanol, an intermediary solvent with a polarity index of 5.2 extracted bioactive phytocompounds responsible for toxicity of mosquito larvae. Ethanol has the property to extract alkaloids, coumarins, flavonoids, phenols, quinines, saponins, sterols, tannins, terpenes and terpenoids which are toxic to the immature mosquitoes (Shaalan et al., 2005).

Samuel et al. (2018) provided an exhaustive review on the list of ethanolic plant extracts reported for mosquito larvicidal property, and in the present study *T. procumbens* ethanolic leaf extract against *Ae. aegypti* and *Ae. albopictus* larvae (first time) has been reported for its toxicity in this study. An examination into the larvicidal mode of action by these phytochemicals on species of *Aedes* mosquito larvae include, direct attack and damage on the nervous system, affect the midgut epithelium primarily, and affect the gastric caeca and the Malpighian tubules secondarily (Rey et al., 1999), act as mitochondrial poison (Mann and Kaufman, 2012) and work by interacting with cuticle membrane of the larvae ultimately disarranging the membrane which is the most probable reason for larval death (Hostettmann and Marston, 1995). In the present study, *T. procumbens* extracts caused mortality to *Ae. aegypti* and *Ae. albopictus* larvae which can be attributed to the presence of its phytochemical compounds, viz., betulin, betulinic acid, lupeol (triterpenoids), caryophyllene, isophytol, phytol (terpenes), limonene (monoterpene), luteolin (flavonoid), stigmast-5-en-3-ol, 3 (beta)-, stigmast-5-en-3-ol, oleate, and palmitic acid (sterols). Thus, *T. procumbens* extracts possess lethal effects against larvae of *Aedes* spp., and this study corroborates the findings of da Silva et al. (2016) who reported on the action of triterpenoids on *Ae. aegypti* larvae. Further, Samuel et al. (2020) reported that the flavonoids, tannins, limonoids (terpenoids) of *Citrus limon* (L.) Osbeck leaf extracts arrested the metabolic activities of *Ae. aegypti* larvae, inhibited its skin changes, disrupted the body's metabolism which resulted in lack of energy for life activities, and caused the *Ae. aegypti* larvae to spasm and eventually in its death. The same was observed in the present study too. Selection of mosquito species for testing is also of fundamental importance since great variations exist in responses between the genera and species, and in the present study Aedines were selected as they are the most commonly colonized mosquitoes which are less susceptible to insecticides.

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EVALUATION OF SEED SOAKING INSECTICIDES AGAINST INSECT PESTS OF SORGHUM

S S Kharbhantanal, Saicharan Dharavath* and H C Latha

Department of Agricultural Entomology, University of Agricultural Sciences Dharwad, College of Agriculture, Vijayapur 586101, Karnataka, India
*Email: charan.dharavath@gmail.com (corresponding author)

ABSTRACT

In this study, seed soaking insecticide treatments i.e., thiamethoxam 25%WG @ 2.0 g/ l, clothianidin 50%WG @ 2.0 g/ l, dinotefuron 20%SG @ 1.0 g/ l, fipronil 5%SC@ 1.0 ml/ l, flonicamid 50%SG @ 0.50 g/ l + $CaCl_2$ @ 2% were evaluated in sorghum variety M 35-1 during 2018 and 2019. The pooled data in terms of deadhearts (DH) showed significant reduction in damage, with least % deadhearts by the shoot fly (*Atherigona soccata* Rondani) (17.96%), and stem borer (*Chilo partellus* Swinhoe) (4.32%); while shoot bugs (*Peregrinus maidis* Ashmead)/ plant (5.88) and number of aphids- *Rhopalosiphum maidis* (Fitch.)/ 3 cm^2 leaf area (23.57) were significantly reduced, along with maximum grain yield (1810 q/ ha) being with thiamethoxam.

Key words: *Atherigona soccata*, *Chilo partellus,* deadhearts, efficacy, *Peregrinus maidis*, *Rhopalosiphum maidis*, sorghum, seed soaking, thiamethoxam

Sorghum (*Sorghum bicolor* [L.] Moench) is the fifth most important cereal crop attacked by nearly 150 insect pest species. (Sharma, 1985). The sorghum shoot fly (*Atherigona soccata* Rondani) (Diptera: Muscidae) causes severe damage in the early stage and lasts up to four weeks. Maximum yield losses of 75.6% in grain and 68.6% in fodder had been reported by Pawar et al. (1984). The stem borer (*Chilo partellus* Swinhole) is also an important pest with about 4-45% deadhearts. Sorghum aphid (*Rhopalosiphum maidis* Fitch.) is distributed in Asia, Africa and Americas. It prefers to feed on the under surface of older leaves, resulting in premature drying of leaves, non-filling of grains and deterioration of fodder quality. The shoot bug *Peregrinus maidis* (Ashmead) is a major pest in rabi sorghum causing dual problem of direct loss by sucking the sap and indirect damage by transmitting sorghum stripe virus disease. Hence, it comes in the way of harvesting potential yield of grain and fodder. The consolidated strategy to manage this pest is combination of cultural practices, natural enemies, insecticides and host plant resistance. Chemical control alone proves expensive as it requires repeated applications against target pest which is not affordable for marginal farmers as well as safety concern to dairy animals (Gahukar, 1991). Therefore, the seed soaking seems to be a viable option for pest management system in terms of cost effectiveness and compatibility with other components of IPM (Balikai, 2011; Singh et al., 2017) and also protection of earlystage growth of the plants which is most susceptible to shoot fly devastation (Balikai and Bhagwat, 2009). This study evaluates different seed soaking insecticides and their cost-effective dose for the protection of most susceptible stage of the crop against attack by *A. soccata* and other sorghum insect pests.

MATERIALS AND METHODS

A field experiment was conducted at the Regional Agricultural Research Station, Vijayapur, Karnataka, during two consecutive rabi seasons of 2017-2018 and 2018-2019 under rainfed conditions in a randomized block design replicated thrice with seven treatments including farmer's practice (T6). The commercial sorghum variety M35-1 was planted at a spacing 45x15cm in a plot size of 10.8m^2, each having six rows. The seeds were soaked in chemicals for 5hr, dried in shade and used for sowing. The thinning of sorghum plants was done a week after emergence of the crop. The total number of plants and number of plants showing dead heart symptoms were recorded in each treatment on 28th day after emergence of the crop. The deadhearts caused by *A. soccata* was worked out and subjected to angular transformations before analysis. The *P. maidis* population was counted on randomly selected five plants in each treatment at 50 days after sowing. The *R. maidis* incidence was recorded when the incidence was at its peak during January second week (80 days after sowing). The data were subjected to angular transformations before statistical analysis. The data

on infestation parameter (DH%), number of *R. maidis*, number of *P. maidis* and yield from individual trials year wise were pooled and analysed using two-way ANOVA as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The efficacy data in terms of deadhearts (DH), number of *R. maidis* and *P. maidis* over two years when pooled and analysed it was observed that reduction in damage by *A. soccata* and *C. partellus* (as % deadhearts-DH) revealed significant differences with seed soaking insecticides. The range of % DH (30DAE) of *A. soccata* was 17.96 to 26.28 and *C. partellus* (45DAE) was 4.12 to 5.70 in all the seed soaking insecticides in comparison to recommended package of practices (RPP) (24.46 and 5.08) and control (40.13 and 7.19) during 2018-19 and 2019-20 (Table 1). The pooled data revealed significantly less DH due to *A. soccata* was observed with thiamethoxam 25%WG @2.0 g/ l (17.96%) and flonicamid 50%SG @ 0.50 g/ l (18.99%), both at par with each other. Similarly, the DH due to *C. partellus* was less in case of flonicamid 50%SG @0.50 g/ l (4.12%) followed by thiamethoxam 25%WG @2.0 g/ l (4.32%). Ravinder Kumar (2018) suggested that seed treatment with thiamethoxam 30 FS @ 10 ml/ kg reduced shoot fly incidence. The reduction in number of *P. maidis*/ plant and *R. maidis*/ 3 cm^2 leaf area revealed similar trend showing the superiority of thiamethoxam 25%WG @ 2.0 g/ l. Sandhu (2016) observed that seed treatment with thiomethoxam 30FS @ 5ml/ kg seed was effective in reducing *A. soccata* incidence; Yue et al. (2003) with European corn borer observed similar efficacy. Daware et al. (2012) concluded that either seed treatment with thiamethoxam 70WS @3g/ kg seed alone or in addition to seed treatment one spray with NSKE @ 5% @ 45 days after crop emergence, could be recommended against major pests of sorghum including shoot fly, shoot bug and aphids. The grain yield (q/ ha) revealed that an yield of 1818 q/ ha was obtained by seed soaking with thiamethoxam 25 WG @2.0 g/ l+ $CaCl_2$ @ 2%. Kumar and Prabhuraj (2007) confirmed the efficacy of seed treatment against *A. soccata*, grain yield and suggested that thiamethoxam 70WS @ 2g/ kg was superior.

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Table 1. Efficacy of seed soaking insecticides against insect pests of sorghum (Pooled data 2018-20)

	Treatment Details	*A. soccata* deadheart at 30 DAE (%)	*C. partellus* deadheart at 45 DAE (%)	No. of *P. maidis*/ plant	No. of *R. maidis*/ 3 cm^2 leaf area	Grain yield (q/ ha)
T_1	Seed soaking in thiamethoxam 25 % WG @ 2.0 g/ l + $CaCl_2$ @ 2%	17.96 (25.07) a	4.32 (11.99) a	5.88 (2.52) a	23.57 (4.91) a	1810
T_2	Seed soaking in clothianidin 50%WG @ 2.0 g/ l + CaCl2 @ 2%	26.28 (30.84) cd	4.27 (11.92) a	9.51 (3.16) a	29.82 (5.51) ab	1493bc
T_3	Seed soaking in dinotefuron 20%SG @ 1.0 g/ l + CaCl2 @ 2%	26.00 (30.66) cd	5.7 (13.81) a	10.40 (3.30) ab	30.47 (5.57) abc	1494bcd
T_4	Seed soaking in fipronil 5%SC@ 1.0 ml/ l + CaCl2 @2%	25.17 (30.11) cd	5.49 (13.55) ab	12.21 (3.57) a	31.76 (5.68) abc	1528
T_5	Seed soaking in flonicamid 50%SG @ 0.50 g/ l + CaCl2 @ 2%	18.99 (25.83) ab	4.12 (11.70) a	7.84 (2.89) a	25.78 (5.13) ab	1665
T_6	RPP: Soil application of carbofuran 3G (25 kg/ ha)- Seed treatment with chloropyriphos 20EC @ 5ml in 20ml of water- Spray of cypermethrin @ 0.50 ml/ l at 45 Days after emergence (DAE)	24.46 (29.64) cd	5.08 (13.03) ab	6.68 (2.68) a	26.27 (5.17) a	1798
T_7	UTC	40.13 (39.31) e	7.19 (15.55) b	19.43 (4.46) b	41.89 (6.51) c	1353d
CD (p= 0.05)		3.25	2.95	0.97	0.91	213.01
S.Em,±		1.08	0.97	0.32	0.30	70.30
CV (%)		11.17	12.67	13.02	12.13	13.45

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FIELD EFFICACY OF REDUCED ACTIVE INGREDIENT ZINC PHOSPHIDE FORMULATION AGAINST RODENTS

Neena Singla*, Rajwinder Singh, Navdeep Kaur and Bhupinder Kaur Babbar

Department of Zoology, Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: neenasingla1@gmail.com (corresponding author)

ABSTRACT

A formulation of zinc phosphide with reduced active ingredient (40% concentrate) was evaluated for its rodenticidal activity in wheat, rice and sugarcane crops at farmer fields of Punjab, India. Comparison was made with the existing formulation (80% concentrate) and second generation anticoagulant bromadiolone (0.25% concentrate). Cereal based baits containing different doses of new (1.5, 2.0 and 2.5%) and existing zinc phosphide (2%) and bromadiolone (0.005%) were applied through burrow baiting where *Bandicota bengalensis, Mus booduga, Millardia meltada* and *Tatera indica* were the predominant rodents. The reduction in rodent activity was determined based on pre- and post-treatment bait census. Results revealed that 2% and 2.5% bait formulations of new zinc phosphide are as effective as the existing/ recommended zinc phosphide (2%) and bromadiolone (0.005%) bait formulations.

Key words: Zinc phosphide, 40% and 80% concentrate, bromadiolone, *Bandicota bengalensis*, *Mus booduga*, *Millardia meltada*, *Tatera indica* cereal bait, rice, rodent burrows, sugarcane, wheat

Rodents belong to most diverse order Rodentia of class Mammalia, with about 2277 species (Burgin et al., 2018; Singla, 2021). They cause direct damage to agricultural produce by feeding, burrowing and hoarding, and indirect loss by contaminating food grains with their fur, urine and faeces thereby spreading diseases of zoonotic importance (Singla et al., 2016; Kaur and Singh, 2019). The existing rodent control strategies include environmental, cultural, mechanical and chemical methods (Anonymous, 2021). Use of rodenticides is the most common, but several considerations are shaping their future with focus on their stability and non-target interference. Currently 2% bait formulation of 80% zinc phosphide and 0.005% bait formulation of 0.25% bromadiolone are recommended (Anonymous, 2021), and zinc phosphide is the most commonly used and forms 80-90% of rodent control operations (Buckle and Eason 2015). But these rodenticides can have negative impacts on non-target animals (Witmer et al., 2013; Thomas et al., 2011; Gabriel et al., 2012). Most of these impacts are attributed to anticoagulant poisoning, but in some cases, these are caused by accidental consumption of zinc phosphide (Muraina et al., 2018). The mortality rate of zinc phosphide poisoning is around 37-100% (Sogut et al., 2011). Also, there is no antidote known for zinc phosphide (Bilics et al., 2020). So, there is a need to recommend zinc phosphide formulation which is safe, less toxic and require more consumption to reach a lethal effect. The present study focuses on this need with determining the efficacy of new safer formulations with reduced active ingredient (40%).

MATERIALS AND METHODS

The present study was conducted in three crops i.e. wheat (*Triticum aestivum*), rice (*Oryza sativa*) and sugarcane (*Saccharum officinarum*) at farmers' fields in villages namely Bagga Khurd, Ladhowal and Bhundri (district Ludhiana), and Bandala (district Jalandhar) of Punjab, India in 2018-19 where rice-wheat is the major cropping system. Six blocks (I-VI), each further consisting of three replicated fields (of 0.4 ha each) were selected for each crop. Fields of wheat crop sown under both conventional tillage and with Happy seeder drill (rice residue incorporated as surface mulch) were selected. In wheat crop sown under rice residue management, rodenticide treatments were done at germination stage during November-December, whereas, in wheat crop sown under conventional tillage and rice crop rodenticide treatments were done before milky grain stage during March and September, respectively. In sugarcane crop, treatment was done during October-November when there is increase in sugar content in the cane. Prior to treatment, live burrow count was recorded in all the fields and the surrounding peripheral area up to 10 m width on all sides by plugging all the burrow holes in the evening and counting the number of reopened burrows in

the next morning (Anonymous, 2021). Burrows of different rodent species were identified on the basis of characteristic burrow entrances (Neelanarayan, 2004). Pre-census bait consumption was recorded by placing 10 g of plain bait (cracked wheat grains smeared with 2% groundnut oil) each at 100 bait points/ ha (1kg/ ha) for two days. On third day, left over bait in each field was collected separately from each field and weighed to record the consumption (g/ 100g bait).

Burrows of blocks I to V were treated with different poison baits twice at an interval of about 15 days and one block (block VI) was kept as untreated control. Burrows of blocks I, II and III were treated with three different bait formulations of 40% zinc phosphide i.e. 1.5, 2.0 and 2.5% prepared by mixing 3.75, 5.0 and 6.25 g of zinc phosphide powder in 96.25, 95.0 and 93.75 g cracked wheat grains smeared with 2% groundnut oil, respectively. Burrows of blocks IV and V were treated with 2% bait formulation of 80% zinc phosphide and 0.005% bait formulation of 0.25% bromadiolone prepared by mixing 2.5 and 2.0 g of rodenticide powders with 97.5 and 98.0g cracked wheat grains smeared with 2% groundnut oil, respectively. All the baits were prepared a fresh depending upon the requirement (10g per burrow). Burrow baiting was done by inserting 10g of poison bait taken in a paper boat 6 inches deep inside each burrow. After 15 days of treatments, post-census bait consumption was recorded in all the treated and untreated fields by placing plain bait for two days. To determine the efficacy of treatment, % reduction in rodent activity was determined as per the method described in Singla et al. (2015). Rodent damage in all the fields was determined at preharvest stage selecting five random samples from four sides and centre of a field. Rodent damage in wheat and rice crops was estimated during April and October, respectively following Singla and Babbar (2010). Rodent damage in sugarcane was estimated during December following Singla and Babbar (2012). The values were determined as mean± SD, and significance of difference in % reduction in rodent activity, cut tillers and yield loss was determined using one way ANOVA (p=0.05).

RESULTS AND DISCUSSION

In wheat crop fields sown under rice residue management at villages Bagga Khurd and Ladhowal, district Ludhiana, burrows of *Bandicota bengalensis, Mus booduga* and *Tatera indica* were found. The reduction in rodent activity after two treatments ranged from 38.8 to 96.4%, being highest in block III treated with 2.5% formulation of 40% zinc phosphide (Table 1). No significant difference was found in reduction in rodent activity between blocks II and III. In blocks IV and V treated with already recommended bait formulations of zinc phosphide and bromadiolone, the mean reduction in rodent activity after two treatments was 82.9 and 76.4%, respectively which was at par with that obtained in block II (77.3%) and III (96.4%) treated with 2% and 2.5% bait formulations of 40% zinc phosphide. Significantly lower mean reduction in rodent activity was obtained in block I (38.8%) treated with 1.5% formulation of 40% zinc phosphide. In untreated block, there was found an increase in rodent activity. Record of rodent damage at preharvest stage revealed significantly low mean cut tillers (0.3 to 0.6%) and yield loss (7.9 to 13.7 kg/ ha) in treatment blocks II to V as compared to block I (1.3% and 30.7 kg/ ha, respectively) and untreated block VI (2.0% and 59.7 kg/ ha, respectively). In wheat sown under rice residue management, double burrow baiting with 2% formulation of 80% zinc phosphide is already recommended during germination stage (Singla, 2019), but it can be replaced with double burrow baiting of 2% or 2.5% formulation of 40% zinc phosphide to reduce non-target toxicity. In wheat crop sown under conventional tillage at villages Bagga Khurd and Ladhowal, district Ludhiana, burrows of *B. bengalensis, M. booduga, T. indica* and *Millardia meltada* were found. The mean reduction in rodent activity in block I treated with 1.5% bait formulation of 40% zinc phosphide was non-significantly low (67.0%) than that observed in blocks II to V (77.3 to 98.0%) indicating its low efficacy. Damage at pre-harvest stage revealed significantly low mean cut tillers (0.7 to 01.3%) and yield loss (17.3 to 23.3 kg/ ha) in treatment blocks II to V as compared to block I (2.3% and 42.4 kg/ ha, respectively) and untreated block VI (6.4% and 105.0 kg/ ha, respectively). No significant difference was found in efficacy of 2% and 2.5% bait formulations of 40% zinc phosphide and 2% and 0.005% bait formulations of 80% zinc phosphide and bromadiolone, respectively (Table 1).

In rice crop fields selected at village Bhundri, district Ludhiana, burrows of three rodent species i.e. *B. bengalensis, M. booduga* and *M. meltada* were found. The reduction in rodent activity after two treatments ranged from 62.7 to 86.7%, in blocks I to V with no significant difference among them. Among the three blocks treated with 40% zinc phosphide, higher reduction (76.0%) in rodent activity was obtained in block III treated with 2.5% bait formulation. In

Table 1. Efficacy of rodenticide treatments in wheat, rice and sugarcane crops

Blocks	Wheat crop						Rice crop		Sugarcane crop	
	Under rice residue management			Under conventional tillage						
	Reduction in rodent activity (%)	Cut tillers (%)	Yield loss (kg/ ha)	Reduction in rodent activity (%)	Cut tillers (%)	Yield loss (kg/ ha)	Reduction in rodent activity (%)	Cut tillers (%)	Yield loss (kg/ ha)	Reduction in rodent activity (%)	Cut tillers (%)
I	38.8± 6.1^{a}	1.3± 0.1^{a}	30.7± 3.4^{a}	67.0± 6.2^{a}	2.3± 0.7^{a}	42.4± 13.1^{a}	62.7± 18.6^{a}	0.6± 0.4^{a}	17.5± 12.0^{a}	22.5± 4.9^{a}	3.1± 0.2^{a}
II	77.3± 6.6^{b}	0.6± 0.1^{b}	13.7± 3.8^{b}	77.3± 8.6^{a}	1.3± 0.4^{b}	23.3± 7.5^{b}	68.3.± 4.2^{a}	0.5± 0.1^{a}	16.9± 4.9^{a}	82.2± 3.1^{b}	1.3± 0.4^{b}
III	96.4± 1.5^{b}	0.3± 0.1^{b}	7.9± 1.7^{b}	87.3± 4.0^{a}	0.7± 0.5^{b}	17.3± 9.7^{b}	76.0± 13.9^{a}	0.4± 0.1	13.9± 0.2^{a}	91.1± 1.7^{b}	0.9± 0.3^{b}
IV	82.9± 2.2^{b}	0.4± 0.1^{b}	9.3± 1.6^{b}	98.0± 1.7^{a}	0.8± 0.3^{b}	18.4± 7.0^{b}	80.0± 6.6^{a}	0.3± 0.1	12.6± 2.9^{a}	79.0± 6.5^{b}	1.0± 0.2^{b}
V	76.4± 2.8^{b}	0.5± 0.1^{b}	11.3± 1.2^{b}	94.3± 0.6^{a}	0.8± 0.2^{b}	18.7± 5.8^{b}	86.7± 1.5^{a}	0.3± 0.1^{a}	10.4± 2.4^{a}	70.2± 5.6^{b}	1.2± 0.2^{b}
VI	Increase	2.0± 0.1^{c}	58.7± 5.0^{c}	Increase	6.4± 2.2^{c}	105.0± 25.1^{c}	Increase	1.2± 0.4^{b}	35.2± 17.4^{b}	Increase	6.0± 1.6^{c}

Values Mean± SD; Values with different superscripts in a column differ significantly at p=0.05

blocks IV and V, the reduction in rodent activity was 80.0 and 86.7%, respectively after two treatments. Record of rodent damage at pre-harvest stage revealed significantly less cut tillers (0.3 to 0.6%) in treatment blocks compared to untreated block (1.2%). Similarly, the yield loss was also significantly low (10.4 to 17.5 kg/ha) in treatment blocks compared to untreated block (35.2 kg/ ha). Cut tillers and yield loss in all the treated blocks were almost similar (Table 1). Singh et al. (2017) recorded 44.47-65.64% reduction in rodent activity in direct seeded and 49.76-61.68% in transplanted basmati rice crops where burrow baiting was practiced with zinc phosphide at vegetative phase.

In sugarcane crop field selected at village Bandala, district Jalandhar, burrows of three rodent species i.e. *B. bengalensis, M. booduga* and *M. meltada* were found. The reduction in rodent activity was significantly low (22.5%) in block I as compared to blocks II to V (70.2 to 91.1%) with maximum reduction in activity in block III treated with 2.5% bait formulation of 40% zinc phosphide. No significant difference was found in per cent reduction in activity among blocks II to V. Record of rodent damage at pre-harvest stage revealed significantly low per cent cut canes (0.9 to 1.3%) in treatment blocks II to V as compared to block I (3.1%) and untreated block VI (6.0%) (Table 1). In sugarcane crop, it is recommended to conduct baiting with 2% zinc phosphide formulation (80%) followed by baiting with 0.005% bromadiolone after 15 days, first in July (during rice transplantation in surrounding fields) and second in October-November (after rice harvest in surrounding crops) (Singla and Babbar 2012). This can be replaced with 2.5% bait formulation of 40% zinc phosphide to reduce non-target effects.

Burrow baiting is recommended during lean period and crop germination stages to avoid non-target toxicity hazards due to less or no crop cover. But still there may be non-target toxicity caused by these rodenticide baits when dogs dig the rodent burrows and throw the bait outside the burrow leading to direct poisoning of non-targets (Muraina et al., 2018) or secondary poisoning when the natural predators consume rodents died after consuming rodenticide bait (Gabriel et al., 2012). Present study conducted in different crops concluded that 2% and 2.5% bait formulations of 40% zinc phosphide are as effective as the already recommended 2% formulation of 80% zinc phosphide and 0.005% bait formulation of 0.25% bromadiolone and can be used for managing rodent pest population in crop fields with reduced risk to non-targets.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS STATEMENT

NS conceived and designed research. RS, NK and BKB conducted experiments. NS and NK analyzed data. NS, RS and NK wrote the manuscript. All authors read and approved the manuscript.

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INTERCEPTION OF LIVE *PHRATORA LATICOLLIS* (SUFFRIAN) (COLEOPTERA: CHRYSOMELIDAE) ON POPLAR LOGS IMPORTED FROM BELGIUM AND GERMANY

Nagaraju D K*, Jain R K, Iyyanar D, Maharaj Singh, Kasturi N, Prema Ranjitham T, Om Prakash Verma and Ravi Prakash

Directorate of Plant Protection, Quarantine and Storage, NH-IV, Faridabad 121001, Haryana, India
*Email: dkn.raju@gov.in (corresponding author)

ABSTRACT

Logs of *Populus nigra* L., frequently exported from Belgium and Germany are frequently being intercepted with a beetle *Phratora laticollis* (Suffrian) (Coleoptera, Chrysomelidae, Chrysomelinae), which is a pest of poplar trees in their native range. Such interceptions of exotic pests pose biosecurity risk to India, and hence these intercepted shipments require to be fumigated with methyl bromide @ 48 g/ m^3 for 24 hr at the normal atmospheric pressure. The treated shipments also need to be re-inspected prior to release to ensure that these are free of live infestation. Its non-compliances need to be notified to the trading partners on each interception as per the guidelines in the ISPM-13. Significance of such interception in plant biosecurity is discussed herein.

Key words: Biosecurity, DIP Act, fumigation, interceptions, invasive insects, IPPC, ISPM, methyl bromide, *Phratora laticollis*, poplar logs, *Populus nigra*, PQ Order, safety matches

Indigenous and cultivated exotic poplar is inadequate to meet the total demand of nearly 2000 match industries in India (Haritha, 2019). Logs of *Populus nigra* L. (Salicaceae) are imported for the manufacturing of splints, a raw material for the safety match industry. Poplar is preferred over other plant species for their colour and quality of splints, and demand for such safety matches is high in the international market. Safety matches made of imported poplar are mostly being exported and those made of indigenous wood such as white mutty *Ailanthus triphysa* (Dennst.) Alston (Simaroubaceae) are sold in the domestic market (Tandon, 1991). Most of the logs are imported through the Tuticorin port in Tamil Nadu, where 90% of match industries are located (Haritha, 2019). This study reports the observations from inspecting and identifying exotic insects associated with logs of *P. nigra* imported from Belgium and Germany.

MATERIALS AND METHODS

The imported logs of poplar *P. nigra* were regularly inspected by the Plant Quarantine Station at Tuticorin as per the provisions of Plant Quarantine (Regulation of Import into India) Order 2003 issued under Destructive Insects and Pests Act, 1914 to ensure freedom from biosecurity risks. During this procedure, adults of a chrysomelid beetle were frequently intercepted from these logs. The beetles were observed feeding on the sprouts of the logs and resting on walls of containers, which flew actively when disturbed. The specimens from the intercepted logs were collected and stored in 70% ethyl alcohol before dissection, and examination. Adult male habitus and genitalia were photographed using a Leica M205C stereozoom microscope. Multiple images taken at different depths were combined using Combine ZM software. The specimens are deposited in the collection of the Department of Entomology, Gandhi Krishi Vigyana Kendra (GKVK), University of Agricultural Sciences, Bangalore (UASB), India.

RESULTS AND DISCUSSION

Intercepted beetles were identified as *Phratora laticollis* (Suffrian, 1851) (Coleoptera, Chrysomelidae, Chrysomelinae) (Fig. 1). It is a pest of poplar native to Europe and hither to unknown in India. In their native range, pest is known to occur round the year and adults overwinter under the bark of trees (Read, 1997). Belgium and Germany are the only exporters of poplar to India, the former being the major exporter. During 2018-2020, 75 of 500 and 3 of 40 consignments from Belgium and Germany respectively, were intercepted with live adults of *P. laticollis* (Table 1). Adults and larvae cause economic damage by feeding gregariously on the ventral side of the leaves (Read, 1997). Inadvertent introduction of *P. laticollis* is detrimental to native *Populus* spp. and other hosts. The association of live adults of any insect in a pathway is a biosecurity risk, as chances of establishments are ensured under suitable

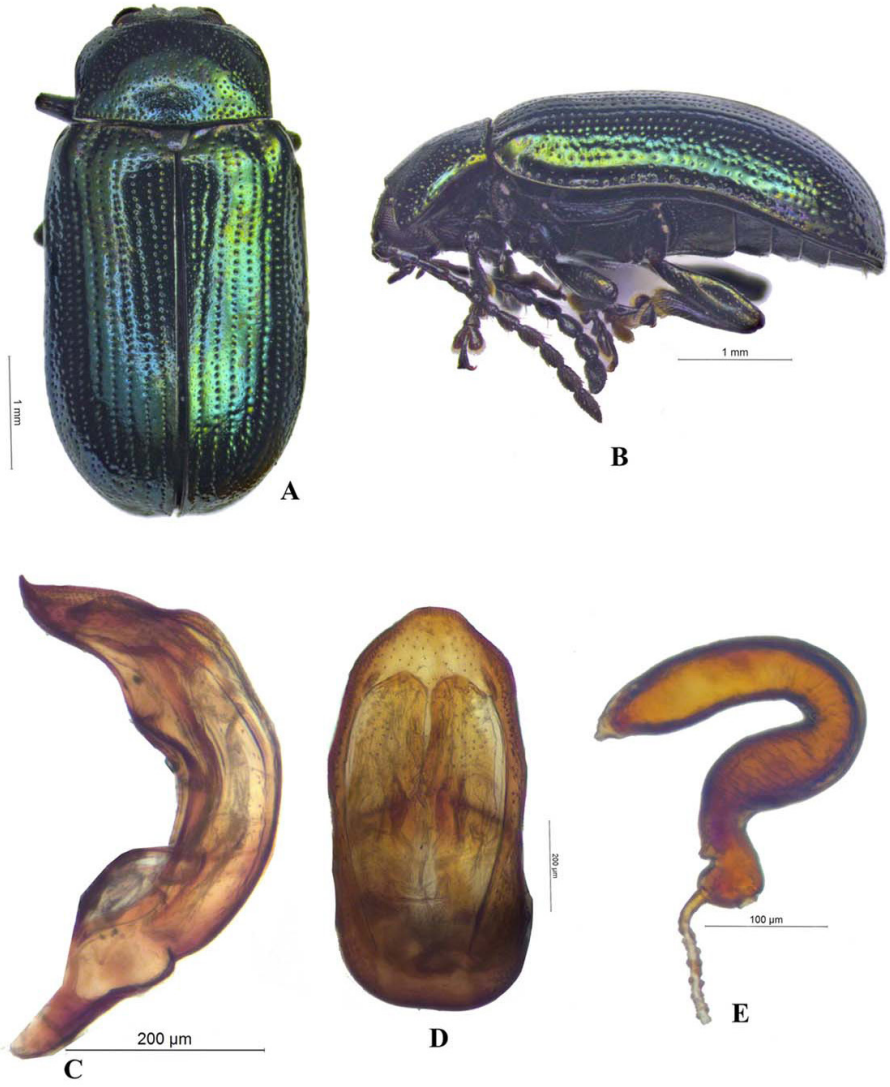

Fig. 1. *Phratora laticollis*. A. Male dorsal view, B. Lateral view, C. Aedeagus in lateral view, D. Apex of Aedeagus, E. Spermatheca

Table 1. Poplar consignments imported and intercepted with *P. laticollis*

Country	Period	Import		Interception (# consignments)
		Quantity (mt)	# Consignments	
Belgium	2018-19	61,608	260	60 (23.08)*
	2019-20	55,711	240	15 (6.25)
		117,319	500	75 (15.00)
Germany	2018-19	4,178	19	02 (10.53)
	2019-20	4,007	21	01 (4.76)
		8,185	40	03 (7.50)

*Figures in parentheses % of interceptions

weather conditions if hosts are available. Adults which were supposed to overwinter under bark were observed active in the container. Factors triggering adults to break overwintering during the voyage is an added advantage for the species to extend its geographical range. This poses a biosecurity threat for the importing country. Interception of live adults warrants instant mitigation measure and risk assessment (Nagaraju et al., 2020).

Poplar is being imported in shipping containers at >40°C with humidity too increasing if logs are freshly cut, which favors condensation of moisture leading to "container rain" or "cargo sweat". The micro-environment and a long voyage of nearly a month are congenial for logs to sprout. New flush and high temperature trigger the adults to break overwintering and become active during the voyage. Such adults may escape to the environment if proper and instant attention is not taken during the inspection. In India, eight species of indigenous poplars occur in high altitudes in the Himalaya and exotic species introduced for experimental purposes occur in Tamil Nadu and neighbouring states (Dhiman, 2016). Furthermore, their cultivation in farmlands and social forestry is a popular program in many states. Willows *Salix* spp. (Salicaceae), crab apple *Malus* spp. (Rosaceae) and elm *Ulmus* sp. (Ulmaceae) are the alternate hosts of *P. laticollis* seen in the north and north eastern states (Bor, 1958; Naithani and Nautiyal, 2012). Inland trading of imported logs facilitates the exotic pest's access to the hosts. Therefore, *P. laticollis* is a potential threat to native poplar species and other economically and ecologically important hosts. All imported containers intercepted were hence fumigated with methyl bromide @ 48 g/ m^3 for 24 hr at normal atmospheric pressure. These logs were re-inspected to ensure pest free status, and its non-compliances notified to the trading partners on each interception as per the guidelines in the ISPM-13 and the consignments were released for use. Thus, *P. laticollis* remains to be a biosecurity threat to India. This is the first report of the species in imported poplar logs in India and elsewhere.

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DIVERSITY OF ODONATA IN A COFFEE ECOSYSTEM

K R Manikandan*, M Muthuswami, N Chitra and M Ananthan[1]

Department of Agricultural Entomology; [1]Directorate of Open and
Distance Learning Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India
*Email: manibscagri@gmail.com (corresponding author): ORCID ID: 0000-0003-4614-3034

ABSTRACT

A total of 419 individuals under 5 families, 10 genera and 10 species of Odonata were observed in the present study on the Odonata from a coffee ecosystem at the lower Palni Hills, Tamil Nadu, India. Among these, the family Libellulidae included six species followed by Euphaeidae (2), and Chlorocyphidae, Coenagrionidae and Aeshnidae (1 each). The dominant species were: *Pantala flavescens* (44.40%) > *Diplacodes trivialis* (22.70%) > *Orthetrum chrysis* (7.40%). *Pantala flavescens* was maximum during northeast monsoon season (50.0%) followed by summer and winter (43.8% each). Margalef index of species richness was maximum (2.00) during winter, and that of Simpson index was maximum (0.75) during south west monsoon. Shannon-Wiener index of dominance was maximum (1.75) during summer. The species were evenly distributed during summer with Pielou's evenness index value of 0.76.

Key words: Odonata, diversity indices, coffee ecosystem, Southern Western Ghats, lower Palni Hills, seasonal abundance, numerical abundance

Western Ghats, one of the mega hotspot centres of the world is endowed with rich biodiversity with its flora and fauna being largely endemic (https://whc.unesco.org/en/list/1342/). The Palni Hills in Tamil Nadu, which are the eastern extension of the Western Ghats, have invertebrate richness and endemism yet to be explored. The present study on the insect diversity in this region (10.12-10.15°N, 77.26-77.33°E), an inventory of Odonata was undertaken during January, 2018 to 2019, covering an area of 2068 km^2. Odonata are known as ecological indicators, and many studies show that certain species exhibit high association with particular habitats (Smith et al., 2007), especially of freshwater habitats (Subramanian and Sivaramakrishnan 2005). These insectivorous insects are biocontrol agents against mosquitoes (Andrew et al., 2008). Indiscriminate usage of pesticides causes the loss of biodiversity of beneficial organisms. Recently, biodiversity in agricultural land has received growing attention (Dudley et al., 2005). Coffee is the most important perennial beverage, especially in Tamil Nadu (Central Coffee Research Institute CCRI, 2018). In the study area of Thadiyankudisai, coffee is cultivated in an area of 13,436 ha, and insect pests are a major constraint. Basic study on Odonata diversity is a prerequisite for the success of any biological control and IPM measure, and hence the present study.

MATERIALS AND METHODS

The present study was carried out at the Horticultural Research Station, Thadiyankudisai (10.29°N, 77.71°E, 1098 masl) from January, 2018 to January, 2019. Survey sites were chosen based on accessibility, covering the Lower Palni hills where coffee is intercropped with silver oak, pepper, avocado, mandarin orange, macadamia nut, Indian coral tree, silk cotton, jack and banana. The research plot's location is in the northern region of Kodavan river and southern region of Thathampara canals. Collection of specimens was done with a specially designed insect net (30 cm dia of the hoop and collection bag length 60 cm) at weekly intervals Species were photographed with a Canon camera, and identification was done following the taxonomic keys (Fraser, 1933, 1934 and 1936). Expertise of Dr K Gunathilagaraj, Dr Subramanian (Zoological Survey of India, SRC, Chennai), Dr R. Arulprakash and Suhirtha Muhil was availed. Identified collections were deposited at the Tamil Nadu Agricultural University (TNAU), Insect Museum, Coimbatore. Relative abundance was calculated by the formula- relative density (%) = (no. of individuals of one species/ no. of individuals of all species)x 100. Species or alpha diversity was quantified using Simpson's diversity index (SDI-Simpson, 1949) and Shannon-Wiener index (Shannon and Weaver, 1949). Margalef index of species richness was calculated (Margalef, 1958) as $\alpha = (S - 1) / \ln (N)$; S= total no. of species, N= total no. of individuals in the sample. Species evenness was calculated using the Pielou's evenness index ($E1 = H' / \ln(S)$; H'=Shannon-Wiener diversity index, S=total number of species in

the sample (Pielou, 1966) with biodiversity calculator. https://www.alyoung.com/labs/biodiversity calculator.html.

RESULTS AND DISCUSSION

A total of 419 specimens of Odonata under 5 families, 10 genera and 10 species was observed, with Libellulidae being more speciose (6 species) followed by Euphaeidae (2), Chlorocyphidae, Coenagrionidae and Aeshnidae (1 each). The most domiant were- *Pantala flavescens* (44.40%) > *Diplacodes trivialis* (22.70%) > *Orthetrum chrysis* (7.40%). *Pantala flavescens* was the maximum during north east monsoon (50.00%) while *Diplacodes trivialis* (26.90%) was in the south west monsoon (Table 1). Margalef Index (2.00) was the highest during winter, and the Simpson index (0.75) was the highest during south west monsoon; Shannon-Wiener Index of dominance (1.75) was maximum during summer. The species were evenly distributed during summer with Pielou's index being 0.76 (Table 2). The dominance of Libellulidae has been previously reported from other parts of the Western Ghats (Subramanian et al., 2008; Koparde et al., 2015). Libellulidae occur commonly in the plains, semi evergreen forests, moist deciduous forests, coastal swamps (Subramanian et al., 2008). *Pantala flavescens* and *D. trivialis* commonly occur in the agroecosystems such as rice (Gunathilagaraj et al., 1999; Arulprakash et al., 2017) and pulses cultivated under dry irrigated conditions (Chitra et al., 2000). Among Zygoptera, *Esme mudiensis* (Coenagrionidae) is the most common in the wetlands (Subramanian et al., 2008) and from rice fields of Coimbatore (Gunathilagaraj et al., 1999), Pattukottai (Arulprakash et al., 2017). Higher diversity and even species distribution during summer may be attributed emergence of 2-3 generations during summer months (Michael and Norma, 2010).

Libellulidae and Gomphidae are well-distributed Anisopterans across Indian subcontinent, with few species restricted to Western Ghats and/or northeast

Table 1. Seasonal abundance of Odonata in coffee ecosystem at Thadiyankudisai

S. No.	Family/ Scientific name	Seasonal abundance				Numerical abundance (Nos.)
		Winter	Summer	SWM	NEM	
1.	Aeshnidae					
	Anax indicus	2	1	3	2	8
2.	Chlorocyphidae					
	Heliocypha bisignata	2	6	2	6	16
3.	Coenagrionidae					
	Esme mudiensis	3	2	1	5	11
4.	Euphaeidae					
	Euphaea fraseri	1	2	3	0	6
	Euphaea cardinalis	3	3	4	3	13
5.	Libellulidae					
	Brachythemis contaminata	7	4	7	6	24
	Diplacodes trivialis	19	21	28	27	95
	Hylaeothemis indica	5	8	6	10	29
	Orthetrum chrysis	8	7	10	6	31
	Pantala flavescens	39	42	40	65	186
	Total	89	96	104	130	419

No- Total number of individuals collected, SWM- South west monsoon, NEM- North east monsoon

Table 2. Diversity indices of Odonata in coffee ecosystem at Thadiyankudisai

Season	Diversity indices			
	Margalef index (α)	Simpson's index	Shannon-Wiener index	Pielou's index
Winter	2.00	0.74	1.71	0.74
Summer	1.97	0.74	1.75	0.76
South west monsoon	1.93	0.75	1.74	0.75
North east monsoon	1.65	0.67	1.50	0.68

India (Fraser, 1934; 1936; Subramanian, 2005). Two species belonging to Libellulidae and 18 species belonging to Gomphidae are known to be endemic to Western Ghats (Subramanian, 2007). A review by Subramanian et al. (2011) points agricultural pollution and urban and industrial development as major threats to Odonata fauna of Western Ghats.

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization: KRM and NC; Experimentation and data collection: KRM, MM and MA; Data curation: KRM and NC; Formal analysis: KRM and NC; Writing-original draft preparation: KRM, MM, NC and MA; Writing: KRM; Review and editing: KRM, MM and NC; Supervision: KRM, MM, NC and MA; Funding acquisition: KRM and MM; Project administration: MM, NC and MA; All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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RECENT ADVANCES AND CHALLENGES IN IMPLEMENTING IPM PROGRAMMES IN THE ENTOMOLOGICAL CONTEXT OF INDIAN AGRICULTURE

Ruchika Geedi and Gadi V P Reddy[1*]

USDA-ARS, Application Technology Research Unit, Horticultural Insects Research Lab, 1680 Madison Ave. Wooster, Ohio 44691, USA
[1]USDA-ARS, Southern Insect Management Research Unit, 141 Experiment Station Rd., Stoneville, Mississippi 38776, USA
*Email: Gadi.Reddy@usda.gov (corresponding author): ORCID ID 0000-0001-6377-0721

ABSTRACT

Integrated pest management (IPM) programmes are based on using multiple methods to maintain nuisance insects below tolerant levels in crop fields. Recent advances in IPM in developed countries have incorporated biological pesticides, microbial products, semiochemicals, and beneficial insects, but few of such programmes have been successfully implemented in developing countries, such as India. Semiochemicals play critical roles as signals in various interspecific and intraspecific interactions between insects and plants, and among interacting insects, plants, and microbes. In India IPM programmes have included mechanical, chemical, cultural, and biological management strategies. However, among these methods, biological management has its own limitations. Indian IPM scientists mostly work on individual crops, assessing damage severity by specific nuisance arthropods and the efficacy of particular management measure. However, very few government institutions or commercial companies are engaged in developing and commercializing either biological pesticides or semiochemicals. Government institutions mostly focus on research on pheromones of the pestiferous Lepidoptera and Coleoptera. Developing IPM programmes requires a clear understanding of crop-plant development, biology and population dynamics of the nuisance organisms, and the chemical and molecular interactions between the two. It also necessarily requires local knowledge of available, prevalent management tactics. Moreover, the IPM programmes have not been widely adopted in developing countries due to lack of proper knowledge and training farmers in efficient IPM practices, the need for more of human labour, and the complexity of IPM practices, all of which impede on the effective implementation of IPM programmes. In this article, we recapture the historical development of IPM efforts in India and ask whether this concept remains suitable to the present-day challenges in crop production. In this review, more specifically, those factors identified as obstacles to the more widespread adoption of IPM and ways of overcoming such barriers are discussed.

Key words: IPM programmes, present status, adoption, barriers, beneficial insects, biological pesticides, challenges, microbials, botanicals products, semiochemicals, severity, insect plant interactions

Agriculture is the world's largest industry, employing more than a billion people and generating 1.3 trillion-dollar worth of food annually. Crop production is vital in the economic development of any country. In India, ≥75% of people depend on agriculture for livelihood (Kataria and Kumar, 2012). Agriculture employs roughly half of India's workforce and contributes to 17% of India's GDP. India is characterized by an immense diversity in climate, topography, flora, fauna, land use, and socioeconomic conditions (Hinz et al., 2020). Numerous studies have found that biodiversity influences the primary productivity of ecosystems and other aspects of ecosystem functioning. It is also experimentally established that the productivity of many terrestrial ecosystems depends on the availability of limited resources such as soil nitrogen, water, CO_2, herbivory, diseases, and disturbances such as fire or drought (Tilman et al., 2012). India has experienced notable increases in agricultural productivity over the last decades (Hinz et al., 2020). A report by the Department of Agriculture, Cooperation and Farmers Welfare (DAV&FW) reported that the food-grain production in India would be 279.51 mt in 2017-2018. The per capita net availability of food grains increased over time. For example, the per capita net availability of edible *Oryza* sp. (Poaceae) was 58.0 kg/ year in 1951, which has increased to 69.3 kg/ year in 2017, but the area under the cultivation of *Oryza* sp. increased from 30.81 m ha to 43.95 m ha (Nelson et al., 2019).

Pestiferous arthropods damage 18-20% of the world's annual crop production, valued at US$ 470

billion (Sharma et al., 2017). However, losses because of pestiferous arthropods are often considerably higher in the Tropics that mostly include developing countries in Asia and Africa, where most of future increases in human population are expected to occur in the next 50 years. In India the highest crop losses are in *Gossypium* (50%), followed by *Sorghum* (30%) and different millets (30%), and *Oryza*, *Zea*, and various oilseeds (each 25%) (Dhaliwal et al., 2015). Farmers through the world actively use pesticides to suppress nuisance insects. Boedeker et al. (2020) reported that 4.1 MT of pesticides were used worldwide in 2017. However, in recent years, with the growing awareness among consumers regarding pesticides residues in crops and their impacts on non-target organisms and human health has caused farmers to reduce the use of pesticides. The use of alternative management practices as in IPM can enhance consumer acceptance and the sustainability of crop-management systems. The IPM programmes must be based on a thorough understanding of the ecology of the concerned organism and its associated natural enemies and their collective interactions with the crop. IPM programmes increasingly validate an understanding of host-plant resistance, smart use of natural enemies, and redesigned agronomic practices (Alwang et al., 2019). In this article, recent advances in IPM are discussed, and the challenges faced by India to implement the newer practices in IPM programmes are explained. Non-chemical IPM approaches are valuable because of the indiscriminate use of chemical pesticides has led to increased crop production costs concurrently with severe harm to the environment, natural enemies, and human health.

Here, the diverse forms of non-chemical approaches, e.g., use of microbial agents, parasitic and predatory arthropods, entomopathogens, antagonistic microbes, endophytic fungi, botanicals, and crop residues with pesticidal properties into three broad categories (Rao and Rao, 2010). First, augmentative biological control is considered, using predatory and parasitic arthropods. Second is the replacement of synthetic-chemical pesticides with either botanicals or microbes. Third, is the efficacy of semiochemicals in the management of nuisance insects.

A. Present status of IPM in India

Presently, the IPM programmes for managing problems caused by nuiscance arthropods emphasize the adoption of cultural, mechanical, biological, and chemical management (Fig. 1), which are collectively

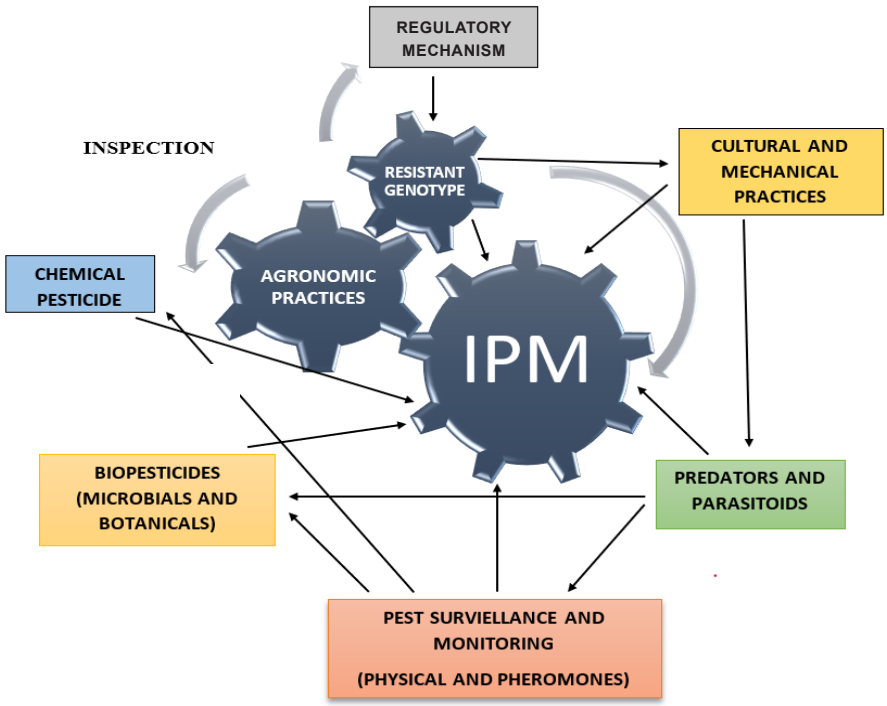

Fig. 1. Schematic diagram of IPM- Black arrows indicate different strategies (physical, mechanical, chemical, cultural and biological controls) associated with IPM modules. The grey arrows represent agronomic practices, resistant genotype, and regulatory mechanisms interconnected with IPM

employed to restrain populations of nuisance arthropods below the economic thresholds (ETLs) (for statistical details, see Bhagat et al., 2016). Most of the Indian farmers (73%) launch nuisance-arthropod-management measures at the time of the first appearance of the concerned arthropod, regardless of the density of the infesting arthropod, developmental stage of the crop, or the interaction patterns between the infesting arthropod and the crop plant (Bhagat et al., 2016). The cost of plant protection on various crops in India range from 7 to 40% of the total crop production cost in the year 2009. Indian farmers see pesticide use as the best means to protect crops from arthropod damage.The synthetic pesticides liberally available in the Indian market include organophosphates, organochlorines, carbamates, synthetic pyrethroids, and neonicotinoids. These are applied both individually and in combination. Although the conventionally used synthetic insecticides usually provide quick and adequate control in the short run, they are expensive (Singh et al., 2012) and pose health hazards, risks of developing resistance in the arthropods thus encouraging tsunamic resurgence of nuisance arthropods, further to environmental pollution (Singh et al., 2012). Using insecticides such as neonicotinoids may also be hazardous to the environment and beneficial arthropods, e.g., pollinators and predators, through direct and indirect exposure (Frank and Tooker, 2020). For instance, the farmers of Vadodara city in India mainly depend on organophosphates (Endosulphan™, Chlorpyriphos™, Parathion™), synthetic pyrethroids (Cypermethrin™, Deltamethrin™), and carbamates (Aldicarb™, Carbaryl™, Carbofuran™) are used for spraying the crops in the agricultural fields for the control of insect pests and to prevent the yield loss.

Although IPM has been advocated in India at least from the 1980s, only 3.2% of Indian farmers have adopted IPM practices in various crops. IPM research has changed Indian farmers' attitudes towards arthropod management in the last decade, resulting in reduction pesticide use by 20–100% in different crops (Rao and Rao, 2010). Effective monitoring of arthropods using traps, a basic measurement tool in the IPM can be achieved by directly sampling the arthorpod or through the use of sex-pheromone traps widely used to monitor populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae), *Spodoptera litura* (Lepidoptera: Noctuidae), *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), *Scirophagous incertulus* (Lepidoptera: Crambidae), species of Dynastinae (Coleoptera: Scarabaeidae), species of *Aproaerema* (Lepidoptera: Gelechiidae) in diverse crop ecosystems (Rao and Rao, 2010). Biorational pesticides include a range of product types with the general traits of being relatively non-toxic, with minimal side effects. These biorational approaches include the monitoring tools (e.g., physical traps, pheromone traps) are effective in indicating the population numbers of nuisance arthropods and thus are useful in applying as conventionally used pesticides as an integrated approach for pest management.

Use of sustainable agricultural practices, such as cultural-control farming, can be manipulated in a variety of ways including either early or delayed sowing, selection of the trap crops, altering plant density or arrangement, sowing genetic mixtures, and improved irrigation methods to reduce the impact of pestiferous arhropods. Farmers in Vadodara prefer the other technique, which involves cultural control practices such as crop rotation and crop residue removal (Kataria and Kumar, 2020). For instance, *Brassica* crops (Brassicaceae) are rotated with non-cruciferous crops, for example, *Cicer arietinum* (Fabaceae) and *Solanum tuberosum* (Solanaceae) to distract pestiferous arthropods, such as *Plutella xylostella* (Lepidoptera: Plutellidae), *Trichoplusia ni* (Lepidoptera: Noctuidae), *Brevicoryne brassicae* (Hemiptera: Aphididae), *Bemisia tabaci* (Hemiptera: Aleyrodidae). Mechanical management includes manual removal of the eggs and larvae of *Earias insulana* (Lepidoptera: Nolidae), *H. armigera*, and *S. litura*. Farmers utilize these simple and common practices to manage pestiferous arthropods. Some farmers prefer to use the high-speed water jets to wash off small insects such as the species of *Aphis* (Hemiptera: Aphididae), species of *Thrips* (Thysanoptera: Thripidae). On the other side, BioLure® (Suterra Monitoring Solutions, USA) have the potential of trapping (around 1000 eggs/trap). In addition, the use of Trichocards™ as a measure of biological management is popular among Vadodara farmers for managing populations of *H. armigera* and *S. litura*. Farmers place Trichocards™ stapled to the abaxial surface of the leaf usually in the mornings, to avoid direct sunlight. These Trichocards™ are released into the fields of species of *Gossypium*, *B. oleracea*, *Ricinus communis* (Euphorbiaceae), where species of *Trichogramma* (Hymenoptera: Trichogrammatidae) parasitise eggs of the infesting Lepidoptera and kill them. Five to eight cards/ha are usually placed, with each card including *Corcyra cephalonica* 1000 eggs. At the time when cards are released into the fields, spraying of insecticides is not recommended (Kataria and Kumar, 2020).

B. IPM challenges and adoption barriers

Implementation of IPM is full of challenges, especially in developing countries. India has 15 agro-climatic regions, based on soil types, rainfall, temperature, humidity, and hydraulics, which influence the cropping systems. The major challenges in implementing IPM programmes and adopting the new techniques in IPM practices are closely linked to policy, social and psychological factors, training and knowledge, and extension methods. The central government should implement a supportive policy for alternative management practices to regulate pestiferous insects. Psychological and social barriers must be carefully considered by farmers and those implementing IPM practices. The delivery of new technologies is crucial and the nature of IPM requires participation calling for a paradigm shift in e xtension methods. IPM implementation also faces the constraints of training and knowledge experienced chiefly by farmers and extension agents. Because of the difficulties in implementing IPM, extension organizations and agencies must play a larger role in educating farmers about the new methods and practices. The DAC & FW in the Union Ministry of Agriculture and Farmers' Welfare promotes IPM approach under the scheme 'Strengthening and Modernization of Pest Management Centres' in 28 states and union territories.The mandate for these centres is pest, disease monitoring, production and release of biological-control agents, conservation of biological-control agents and human-resource development in IPM by imparting training to agricultural extension officers and farmers at the grassroot level by organizing Farmer's Field School.

The Central Integrated Pest Management Centres (CIPMC) in different parts of India are involved in implementation of various eco-friendly plant-protection approaches approved by the Government of India. The CIPMCs carryout various tasks assigned to them periodically to promote sustainable plant-protection approaches. They are conducting season- long training programme of 30 days on IPM and popularizing IPM among farmer community on an annual basis. There are mechanisms to evaluate the success of programmes implemented by the CIPMCs centers. These extensions programmes play a key role in educating farmers about the ecology of pestiferous arthropods and new techniques developed in IPM. The extension organizations cannot address the difficulties of IPM on their own, since they require assistance from other stakeholders. The other challenges include a lack of awareness and innovation among extension personnel and target groups, insufficient cooperation between research and extension agencies, problem of timely and adequate supply of quality inputs, including biocontrol agents and biopesticides, complexity of IPM vs simplicity of chemical pesticides, dominant influence of pesticide industry, non-availability of location specific IPM modules for many crops.

Most of the private, corporate enterprises do not support shifting away from chemical pesticides to biopesticides because use of biopesticides would be less remunerative to them. Public-sector enterprises now hold only 2% of the biopesticide market share. To take advantage of new prospects and address global environmental concerns, the commercial sector needs to transition to biopesticides.The wide adaptability of IPM still remains a question, because of its acceptability in field conditions. Taking economic returns into account of IPM, farmers are confused, whether to accept it or not. To promote IPM, it is necessary to have many field demonstrations offered at the farmer's level. There is hardly any data available on the adoption of IPM in India. According to biopesticide production figures, IPM is predicted to cover 19% fungicides and 17% herbicides of gross cultivated area under IPM.

Resistance to change is widely prevalant in accepting IPM. Biopesticides are slow in action compared with chemical pesticides. Farmers may find it challenging to manage IPM on their own. If IPM needs to be promoted, it would be better to promote it as a community-centric method. Community-centric approach should be followed in India for a better appreciation and wider adoption of IPM. The essential requirements for implementing IPM are as follows: the availability of location-specific IPM modules, which are ecologically sound, economically viable and socially acceptable, area-wide dissemination strategy, high level of target group participation, removal of obstacles in the dissemination of IPM, measuring, evaluating, and publicizing the impacts of IPM. The conservation of natural enemies of pestiferous arthropods and their augmentation is of prime importance. Besides, the intrinsic properties of renewability, reversibility, and resilience of botanicals and biopesticides make them the most dependable tools for sustainable IPM. Hence, to maintain ecological balance and concurrently to manage pestiferous arthropods within thresholds, the use of bio-agents and biopesticides/botanicals should receive priority attention.

C. Augmentative use of predators and parasitoids

Several IPM programs opted in India include the release of insectary-reared parasitoids, or predators in agricultural fields to manage pestiferous insects. The Coccinellidae are the most useful predators in the IPM context. More than 5,200 species have been described worldwide in the Coccinellidae (Boopathi et al., 2020; Hodek et al., 2012). About 90% of approximately 4,200 species of the Coccinellidae are beneficial because of their predatory behaviour, mostly against the Hemiptera and Acarina (Awasthi et al., 2013). Biological management is a sound substitute for toxic insecticides, because biological management not only protects plants, but human beings and the environment as well (Boopathi et al., 2020). There are several habitats where the Coccinellidae occur (Hodek et al., 2012). They feed on sap sucking arthropods such as Aphidoidea, Coccoidea, Thysanoptera, and Acarina (Boopathi et al., 2020).

Harmonia axyridis Pallas (Coleoptera: Coccinellidae) is a popular biomanagement agent for greenhouse pestiferous insects, such as the species of *Aphis*, *Thrips* and *Bemisia*, but has become a serious invasive (Ukrainsky and Orlova-Bienkowskaja, 2014). Sporadic occurrence of *H. sedecimnotata* (Coleoptera: Coccinellidae) has recently been reported in India (Boopathi et al., 2020). Most of H. sedecimnotata occur on *Abelmoschus esculentus* (Malvaceae), *Solanum melongena* (Solanaceae), *Capsicum annuum* (Solanaceae), *Solanum lycopersicum* (Solanaceae), *Psidium guajava* (Myrtaceae) (Boopathi et al., 2020). The releases of the Coccinellidae are used to manage various species of the Aphidoidea infesting species of *Gossypium*, *S. tuberosum*, and *S. melongena* (Long and Finke, 2014). Polyphagous and predatory Coccinellidae who indiscriminately feed on diverse Aphidoidea on plants such as *A. esculentus*, *S. melongena*, *C. annuum* exhibit variations in fitness, and are therefore likely to vary in their genotypes. An important source for biological control agents will be the most effective genotypes of a predator species with a high predation potential (Boopathi et al., 2020). Boopathi et al. (2020) reported that inoculative release of 30-50 adults (both males and females)/ 100 m^2 achieved a reduction up to 90%, of the infesting Aphidoidea. Thus, it may be recommended the release rate of 40 adults/ 100 m^2 to suppress *Aphis gossypii* populations on *S. melongena*. *Harmonia sedecimnotata* is, therefore, a highly promising biological management agent for *A. gossypii* populations that can be achieved for rapid management through inoculative release of adults. Factors that affect the ability of releases of the Coccinellidae to result in rapid reduction in populations of the Aphidoidea in greenhouses include either repeated releases or increased numbers released predatory adults (Riddick, 2017).

Predators used augmentatively for biological management include different species of *Coccinella* (Coleoptera: Coccinellidae), *Chrysoperla* (Neuroptera: Chrysopidae), Staphylinidae (Coleoptera), Syrphidae (Diptera), Reduviidae (Hemiptera), and *Phytoseiulus persimilis* (Mesostigmata: Phytoseiidae).The other success stories in biological management include the management of *Pyrilla perpusilla* (Hemiptera: Lophopidae) was the utilization of egg parasitoids such as *Tetrastichus pyrillae* (Hymenoptera: Eulophidae) and ectoparasitoids *Epipyrops melanoleuca* (Lepidoptera: Epipyropidae) in subtropical india (Gangwar et al., 2008). *Eriosoma lanigerum* (Hemiptera: Aphididae) and *Quadraspidiotus perniciosus* (Hemiptera: Diaspididae) inflict damage to different species of *Malu*s (Rosaceae) plantations and have been successfully controlled by their biological management agents. *Aphelinus mali* (Hymenoptera: Aphelinidae), *Syrphus confrater* (Diptera: Syrphidae) and *Chrysopa scelestes* (Neuroptera: Chrysopidae) have been highly useful in regulating populations of *E. lanigerum*, whereas *Encarsia perniciosi* and species of *Aphytis* (Hymenoptera: Aphelinidae), *Chilocorus bijugus* (Coleoptera: Coccinellidae) were relevant in the instance of *Q. perniciosus*. *Ceratovacuna lanigera* (Hemiptera: Aphididae) was successfully managed by applying *Dipha aphidivora* (Lepidoptera: Pyralidae), species of *Chrysoperla*, diverse Coccinellidae (Coleoptera) and Syrphidae (Diptera); and various arachnids (Araneae) were not helpful in the states of Maharastra and Karnataka in 2003-2004. *Helicoverpa armigera*, a polyphagous pestiferous arthropod, was successfully managed with the use of nuclear polyhedrosis virus (NPV) on species of *Gossypium*, *Phaseolus*, and *Glycine* (Fabaceae), and *C. annuum* in India (see weblinks of DPPQS, Government of India, 2005).

Trichogramma chilonis and *T. japonicum* (Hymenoptera: Trichogrammatidae) are widely used in India presently to manage various Lepidoptera that attack *Oryza sativa* and *Saccharum officinarum* (Poaceae), various species of *Bracon* (Hymenoptera: Braconidae), *B. hebetor*, *B. brevicornis*, *Chelonus blackburnii* (Hymenoptera: Braconidae) to regulate populations of several pestiferous Lepidoptera, such

as *Earias vitella* (Lepidoptera: Nolidae), *Phthorimaea operculella* (Lepidoptera: Gelechiidae), *P. gossypiella* and *H. armigera* on species of *Gossypium*, *S. tuberosum,* and many other plants. *Goniozus nephantidis* (Hymenoptera: Bethylidae) is widely used to manage populations of *Opisina arenosella* (Lepidoptera: Xyloryctidae). *Goniozus nephantidis* is being mass multiplied and released in Karnataka and Kerela. Parasitoids include various species of the Tachinidae (Diptera) and other Hymenoptera, e.g., *Acerophagus papayae* (Encyrtidae) that are being used to regulate populations of *Paracoccus marginatus* (Hemiptera: Pseudococcidae). Shendage and Sathe (2015) have reported that many Tachinidae attack close to 20 pestiferous insects in Kolhapur region. However, no culture method for commercial mass production of the Tachinidae is available in India. Therefore, the augmentative application of the Tachnidae in pestiferous arthropod management is limited. A maximum of 40% and minimum of 2% parasitism was recorded on *S. litura* and a species of *Forficula* (Dermaptera: Forficulidae) and by Tachinidae macrotype egg parasitoids, respectively. For instance, species of *Exorista* (Diptera: Tachinidae) parasitize *H. armigera* and *S. litura*. *Exorista larvarum* (Diptera: Tachnidae) is a Palaearctic species widely distributed in several Asian regions. About 15 lepidopteran families are known hosts for *E. larvarum*. *Exorista japonica* occurs widespread from India to East Asia, and 18 lepidopteran families are recorded as its hosts. The known natural hosts for both species belong mainly to the Lymantriidae, Lasiocampidae, Noctuidae and Arctiidae (Dindo and Nakamura, 2018). *Aphidius colemani* (Hymenoptera: Braconidae) is a solitary, koinobiont endoparasitoid of the Aphidoidea, and is one highly sought after agent to manage pestiferous arthropods that infest greenhouse plants. A natural parasite, *A. colemani* is mainly used to regulate the economically important *Myzus persicae* (Hemiptera: Aphididae) and *A. gossypii*. These Aphididae are highly polyphagous and attack a wide range of vegetable and ornamental crops especially in greenhouses. *Aphidius colemani* can maintain the Aphididae populations at levels similar to those resulting from pesticide applications (Pardo et al., 2015).

Augmentative release of the parasitic Hymenoptera in greenhouses has been used in different parts of the world (Fahrat and Dharmadhrt, 2021). *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae), *A. matricariae* Haliday and *A. ervi* Haliday (Hymenoptera: Braconidae) have been identified to parasitize *M. persicae* infesting *C. annuum* (Fahrat and Dharmadhrt, 2021). Khan et al. (2020) have reported that more than one species of *Trichogramma* are effective biological control agents, functioning as egg parasitoids. It is a fact that these parasitoids were exploited for controlling the several pestiferous insects belonging to the Coleoptera, Hymenoptera, and Lepidoptera. More than 240 species are known, of which 45 are recorded from India. The successful implementation of an augmentative biological control programme requires a thorough understanding of the biology of the pestiferous arthropod, its natural enemies, the crop environment (including other nuisance organism management practices). The success of this approach is dependent on many considerations, that may necessitate modifications of current production practices and management practices.

D. Microbial and botanical pesticides

Biological control offers a better alternative to synthetic chemical pesticides, because biopesticides of either microbial or botanical origin are target specific, with easy biodegradability, shorter life-span, and user friendly in sustainable agriculture (Chattopadhyay et al., 2017). About 100 species of bacteria are presently known as exo- and endo-pathogens of arthropods (Chattopadhyay et al., 2017). But only a few of them are commercially available (Chattopadhyay et al., 2017). Multiple activities of biopesticides are now considered under integrated crop management (ICM). For example, *Serratia entomophila* AB2 (Enterobacteriaceae) reported from epizootic species of *Heliothis* exhibited both fungicidal and nutrient-solubilizing ability (Chattopadhyay et al., 2017). In a few instances, nuclear polyhedrosis virus (NPV) is used as target-specific products, such as NPV for *H. armigera* (HaNPV) and *S. litura* (SlNPV) used to regulate populations of *H. armigera* and *S. litura* in *Gossypium* (Mondal et al., 2021).

Biopesticides are an essential component of IPM programmes to manage pestiferous arthropods thriving on several economically important fruits and vegetables. Globally there were approximately 700 products in this category, based on 175 different active ingredients (Mishra et al., 2018). In India, 15 biopesticides are currently registered of which five bacteria and include *Pseudomonas fluorescens* (Pseudomonadaceae) and four species of *Bacillus* (Bacillaceae), three fungi which include two species of *Trichoderma* (Hypocreaceae) and a species of *Beauveria* (Cordycipitaceae), and two are NPV against *H. armigera* (HaNPV) and *S. litura* (SlNPV), and two include botanicals from *Azadirachta*

Table 1. List of commercially available biopesticides registered in India (Anonymous, 2014)

Biopesticide	Microorganism	Useful in the management of
Bacillus thuringiensis var. *israelensis*	Bacterium	*Plutella xylostella*
Bacillus thuringiensis var. *kurstaki*	Bacterium	*Plutella xylostella*
Bacillus thuringiensis var. galleriae	Bacterium	*Helicoverpa armigera*
Bacillus sphaericus	Bacterium	*Plutella xylostella*
Bacillus firmus	Bacterium	*Plutella xylostella*
Bacillus subtilis	Bacterium	*Diabrotica virgifera*
Trichoderma viride	Fungus	Species of *Fusarium*
Trichoderma harzianum	Fungus	*Fusarium oxysporum*
Pseudomonas fluorescens	Bacterial/ Fungal	Species of *Bemisia*
Beauveria bassiana	Entomopathogenic fungus	*Idioscopus clypealis* Species of *Phenococcus* and *Maconellicoccus* Species of *Hypothenemus*
Paecilomyces lilacinus	Fungus	Species of *Meloidogyne*
Verticillium lecanii	Fungi	*Bemisia tabaci, Myzus persicae*
Verticillium chlamydosporium	Nematophagous fungus	Species of *Meloidogyne*
Metarhizium anisopliae	Entomopathogenic fungus	*Spodoptera litura,* species of *Coptotermes*, *Odontotermes*
NPV of *Helicoverpa armigera*	Virus	*Helicoverpa armigera* living on *Cicer arietinum*
NPV of *Spodoptera litura*	Virus	*Spodoptera litura*
Neem based biopesticides	Plant product	Species of *Bemisia*
Cymbopogon	Plant product	*Agrotis ipsilon*
Heterorhabditis bacteriophora	Entomopathogenic nematodes	Speies of *Leptinotarsa*
Species of *Trichogramma*	Egg parasitoid	Species of *Diatrea*
Fenpyroximate	Pyrazole acaricide	*Tetranychus urticae*

indica (Meliaceae) and *Cymbopogon flexuosus* (Poaceae) (Mishra et al., 2018) (Table 1). Among biopesticides, those including *Bacillus thuringiensis* (Bt) (Bacillaceae), *Trichoderma viride* (Hypocreaceae), species of *Metarhizium* (Clavicipitaceae)*, Beauvaria bassiana* (Cordycipitaceae)*,* and various nuclear polyhedrosis viruses (NPV) (Baculoviruses) affecting insects, predominately the plant-damaging Heterocera and Rhopalocera (Lepidoptera). Microbes such as *Lecanicillium lecanii* (Cordycipitaceae), *Paecilomyces lilacinus* (Ophiocordycipitaceae), *Pochonia chlamydosporia* (Clavicipitaceae), *Nomuraea rileyi* (Clavicipitaceae) are considered entomopathogenic fungi, registered with the National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. Azadirachtin and pyrethrins are widely used. Pyrethrin from *Tanacetum cinerariifolium* (Asteraceae) is known for its potent insecticidal and repellent activity but is relatively less known for use in plant protection (Kachhawa, 2017). Ramasamy et al. (2020) evaluated different microbials useful as pesticides and neem-compounds either singly or in combination (sequential application) against major insect pests such as *P. xylostella* on *Brassica juncea* (Brassicaceae) in different provinces of Cambodia, suggesting that they might be compatible with other plant protection options such as biopesticides viz., fungicides and bactericides.

The economic feasibility and environmental compatibility of microbial or botanicals as tools facilitating sustainable agriculture is well demonstrated presently (Fenibo et al., 2021). Consequently, the use of conventional pesticides in commercial farming is attracting regulatory restrictions leading to 2% decline/ year in synthetic pesticide use in favour of 10% increase in biopesticides as alternative agrochemicals (Fenibo et al., 2021). Microbial pesticides, biochemical pesticides, and plant incorporated protectants (PIPs) are the well known categories of biopesticides, and they fill 5% share of the pesticide global market, with microbial biopesticide taking the lead (Fenibo et al., 2021). Muzemu et al. (2011) reported more than 50% reduction

of *B. brassicae* and *Tetranychus evansi* (Acarida: Tetranychidae) populations by applying leaf powder extract of *Lippia javanica* (Verbenaceae) and *Solanum campylacanthum* (Solanaceae) to replace pesticides by 100% and go for total adoption of biopesticides. However, total adoption of biopesticides is hindered by short supply of products, high cost, and slow action. These drawbacks are mostly offset by the tolerable toxicity, if any, that is displayed by biopesticides. They are also biodegradable, target specific, and can counter pestiferous insects' resistance that generally arise when synthetic pesticides are used (Fenibo et al., 2021).

Microbial insecticides include a microorganism, which could be either a bacterium or a fungus or a virus or a protozoan or an alga as the active component (Kachhawa, 2017). Microbial insecticides can help in controlling many pestiferous arthropods, although each active microbe is usually specific for a target organism (Vikas et al., 2014). For example, *B. bassiana* is an entomopathogenic fungus that kills *H. armigera*, *S. litura*, *P. xylostella*. *Lecanicillium lecani* (Cordycipitaceae) is used to manage populations of *B. tabaci*. The most widely known microbial pesticides are those developed involving strains of *Bt*, which can manage insects attacking *B. oleracea*, *S. tuberosum*, species of *Gossypium*, *Zea mays* (Poaceae), *Nicotiana tabacum* (Solanaceae) and *Glycine max* (Fabaceae) via Cry proteins. To ensure that microbial pesticides do not affect the non-target species, they need to be regularly monitored. Bt-based pesticides are presently considered a crucial component in IPM programmes.

Entomopathogenic fungi such as *B. bassiana* and species of *Metarhizium* are useful in controlling pestiferous Aphidoidea, such as *M. persicae* and *A. gossypii* (Vu et al., 2007), Termitoidea viz., species of *Odontotermes* (Isoptera) (Ambele et al., 2020) and the Lepidoptera viz., *S. litura* (Malarvannan et al., 2010). Nuclear polyhedrosis viruses could possibly used to regulate populations of critical and major pestiferous arthropods such as *H. armigera* and species of *Spodoptera*. da Costa et al. (2019), isolated three isolates of nucleopolyhedrosis viruses from *H. armigera* and compared them genetically and biologically to Gemstar® (polyhedral occlusion bodies of the nuclear polyhedrosis virus of *H. zea*). They reported the genetic sequencing of lef-8 and lef-9 genes, which revealed that the Brazilian isolates were closely related to the nucleopolyhedrosis virus from Australia, South Africa, China and India. The isolates inflicted high rates mortality in third instar larvae of *H. armigera*. The high degree of relatedness among the Brazillian *H. armigera* virus isolates and those of Australia (HearNPV-Aus), China (HearNPV Complete Genome), and South Africa (HearNPV-Nng-1) suggest the highly specific baculovirus infecting *H. armigera* globally. *Bt* is widely used against pestiferous arthropods infesting species of *Gossypium* and vegetable crops such as *S. melongena*, *S. tuberosum*, and *B. oleracea*. Various genetically modified plants, such as *Bt* cotton, corn, tabacco, soybean, maize that produce *Bt* proteins enable the microbial pesticides widespread commercial use. RNAi is a novel and potential tool to develop further pestiferous insect management, targeting various orders of insects including Diptera, Coleoptera, Lepidoptera, Hymenoptera, and Isoptera. Nitnavare et al. (2021) reported that dsRNA are effective against the pestiferous beetles such as *Diabrotica virgifera virgifera* (Coleoptera: Chysomelidae).

Various techniques for an effective oral administration of dsRNA into the gut of insects have been explored. Another technology is the use of nanoparticles as carrier of dsRNA through to insect-gut epithelia. Several nanoparticle systems have been used for this purpose including liposomes, chitosans, and branched amphiphilic peptide capsules (BAPCs) in the Lepidoptera, Coleoptera, Diptera, and Dictyoptera. Silencing efficacies vary among the insect classes wherein the Coleoptera often exhibit 100% susceptibility and the Hemiptera exhibit lesser susceptibility. Lepiodoptera are the most recalcitrant to oral RNAi owing to their highly alkaline gut. These techniques are being gradually accepted in India presently the host-delivered amiRNA-mediated silencing *HaAce1* gene is used for *H. armigera* management (Saini et al., 2018). One limitation of RNAi as a pestiferous insect-management tool is that the target insect must consume a significant dose of dsRNA to be killed; as a result, delivery systems that make such acquisitions possible must be created (Isman, 2019). India has adequate facilities to focus on the development of a product based on nanotechnology based RNA editing and CRISPR/Cas9 mediated genome editing. Several transgenic plants were developed in India, such as *G. max*, *Z. mays*, species of *Gossypium*, and *Brassica napus*. Shelton (1999) worked on the first field release of a genetically engineered insect virus for insect control. In 2017, cooperating with colleagues at the UK-based Oxitec Limited, the first release of a genetically engineered pestiferous insect, *P. xylostella* strain (OX4319L) with a self-limiting gene to control the spread of *P. xylostella* was conducted. In 2015-2020, Shelton was involved

with the introduction of *Bt S. melongena* in Bangladesh and the Philippines. A recent study confirmed that *Bt S. melongena* dramatically reduces insecticides, and that growers receive an average of 19.6% higher yield and 21.7% higher revenue than non-*Bt* varieties. On a per tonne basis, the revenue benefit of using *Bt S. melongena* was 1.7% reflecting different level of acceptability among trade buyers and consumers. Some farmers were prepared to pay higher prices for *Bt S. melongena,* because the fruit was less damaged while others paid a price discount because the *Bt S. melongena* was not available in preferred local varieties. Furthermore, the study confirmed that *Bt S. melongena* is accepted in the market (Shelton et al., 2020); but an idea of introducing and cultivation *Bt S. melongena* was summarily rejected by Government of India in 2010 after several public debates.

Trichoderma species (Hypocreaceae) have been widely used in agriculture. For instance, *T. harzianum* and *T. viride* are the widely used species in India and have been exploited on about 87 different crops. *Trichoderma* acts directly as an entomopathogen through parasitism and the production of insecticidal secondary metabolites, antifeedant compounds and repellent metabolites (Poveda, 2021). The efficacy of different species of *Trichoderma* as an entomopathogen and their effects on various arthropods is summarized in Table 2. On the other hand, the species *T. viride* and *T. citrinoviride* have been reported with the ability to produce different compounds with antifeedant activity against different insects. For example, *T. viride* used to control the *Bombyx mori* (Lepidoptera: Bombycidae), *Corcyra cephalonia* (Lepidoptera: Pyralidae), *H. armigera* and the volatile organic compounds (VOCs) produced is the chitinase. *Trichoderma citrinoviride* used to control the *Schizaphis graninum* (Hemiptera: Aphididae), *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) and the VOCs produced are citrantifidiene, citrantifidiol and bisorbicillinoids that are capable of repellence. Furthermore, several species of *Trichoderma* (*T. harzianum*, *T. viride* and *T.*

Table 2. Efficacy of species of *Trichoderma* (Hypocreaceae) on pestiferous insects

Ecology	Species	Pestiferous insects	Mortality (%)	References
Parasitic	*T. longibrachiatum, T. harzianum*	*Bemisia tabaci*	90% mortality in 14 days	Zahran et al., 2017
		Oryctes rhinoceros	90% mortality in 14 days	Nasution et al., 2018
		Acanthoscelides obtectus	90% mortality in 14 days	Rodriguez-Gonzalez et al., 2020
		Xylotrechus arvicola	90% mortality in 14 days	Rodriguez-Gonzalez et al., 2017
	T. album	*Rhyzopertha dominica*	94% mortality in 7 days	Mohamed and Taha, 2017
	T. longibrachiatum	*Leucinodes orbonalis* (Lepidoptera: Crambidae)	Increase crop yield and causing up to 50% mortality	Ghosh and Pal, 2016
Secondary metabolites	*T. atroviride*	*Drosophila melanogaster*	Prevent feeding and effect the development and survival of larvae	Hernandez et al., 2019
Peptaibols	*T. harzianum*	*Tenebrio molitor*, *Tribolium castaneum, Schizaphis graminum*, *Diuraphis noxia A. gossypii, Amrasca biguttula biguttula*	Mortality rates of 100% in 15 days	Ganassi et al., 2001; Rahim and Iqbal, 2019; Nawaz et al., 2020
Secondary insecticidal metabolites	Species of *Trichoderma*	*Locusta migratoria, Earias insulana, Pectinophora gossypiella*	Mortality percentage reduced to 50% in 3 days	El-Massry et al., 2016

citrinoviride), whose VOCs act as repellants for pestiferous insects, have scope for reducing damage to plants.

Microorganisms based pesticides and their by-products are widely used in pestiferous insect management tactics because they are effective, species-specific and environmentally friendly (Koul, 2011). The microbial biopesticide market constitutes about 90% of total biopesticides including *Bt* in GM plants, and there is ample scope for further development in agriculture. However, there are challenges as well (Koul, 2011). There are at least 1500 naturally occurring insect-specific microorganisms, of which 100 are considered insecticidal (Koul, 2011). More than 200 microbial biopesticides are available in 30 countries affiliated to the Organization for Economic Cooperation and Development (Koul, 2011). There are 53 microbial biopesticides registered in the USA (2006), 22 in Cananda and 21 in the European Union (Skula et al., 2019); although reports of the products registered for use in Asia are lacking (Skula et al., 2019).

Plant-derived products in some instances have been shown to be effective against certain pestiferous insects (Tembo et al., 2018). Several hundred candidate plant species and compounds are now known to have pesticidal properties against a range of pestiferous arthropods. Despite this growing body of information, only a few natural products are commercialized in pestiferous-arthropod management (Tembo et al., 2018). Tembo et al. (2018) reported that plant extracts were used to manage pestiferous insects of various legumes without harming beneficial arthropods. Botanical pesticides repel pestiferous arthrtopods, modify insect behaviour, and may include antifeedant compounds (Singh et al., 2012). In addition, extracts from *Origanum vulgare*, *Thymus vulgaris* (Lamiaceae) and *Trachyspermum ammi* (Apiaceae) showed broad spectrum of antifungal activity against *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Bhavya et al., 2020). Leaves of the species of *Eucalyptus* (Myrtaceae) include many terpenoids such as α- and β-pinene, 1,8-cineole (CIN), terpineol, and globulol, which are useful as fumigants against some stored grain and other pestiferous insects (Fatemeh and Moharramipour, 2017). Botanical insecticides are currently used to control certain pests and details are metioned in Table 3.

E. Semiochemicals used in IPM

Semiochemicals are popular and well accepted in developed countries, but only 7-10 pheromonal lures are presently available in India. Semiochemicals are organic compounds produced by either insects or plants that transmit chemical messages within or between populations. Insects detect semiochemicals from the air because of olfactory receptors on their antennae. As a broad group, semiochemicals include pheromones and allelochemicals (that include kariomones). Pheromones are further divided based on the responses they elicit as sex, alarm, aggregation, and

Table 3. Botanicals used currently to manage arthropods

Botanical	Plants	Arthropods	References
Pyrethrins (pyrethrum, pyrenone)	*Chrysanthemum cinerariaefolium*	*Frankliniella occidentalis*	Yang et al., 2012
Rotenone	Species of *Lonchocarpus, Derris*	*Spodoptera litura*, *Plutella xylostella*	Zubairi et al., 2016
Nicotine	Species of *Nicotiana*	*Grapholita molesta*	Sarker and Lim, 2018
Veratine	*Schoenocaulon officinale*	*Scirtothrips citri*	Godfrey et al., 2005
Ryanodine	*Ryania speciosa*	*Leptinotarsa decemlineata,* Species of *Corythucha*, *Aphis*, *Anasa*	Souto et al., 2021
Limonene	Species of *Citrus*	Species of Pseudococcidae	Hollingsworth, 2005
Neem-based formulations (Small-scale formulators)	*Azadirachta indica*	*Melanotus communis*	Humbert et al., 2017
Neem oil (Bioactive i.e., Limonoids, Nimbin, Salannin, Nimbinin)	*Azadirachta indica*	*Tribolium castaneum, Sitophilus zeamais*	Kumar et al., 2022
Neem seeds, Neem fruit powder extract (NFPE)	*Azadirachta indica*	*Plutella xylostella,* species of *Aphis*	Rao and Rao, 2010

trail pheromones (Fig. 2). Kairomones are chemicals whose detection is advantageous to the receiver, but not to the emitter. Kairomones guide arthropod predators and parasitoids to their hosts or prey. These semiochemicals are used in various insect control strategies such as in monitoring, in mass trapping, to attract and kill approach, and in mating disruption, and as feeding deterrents (Heuskin et al., 2011). Repellants such as verbenone ($C_{10}H_{14}O$), an insect phereomone analogue occurs in a variety of plants, but more commonly in *Salvia rosemarinus* (Lamiaceae), and *Aloysia citriodora* (Verbenaceae) can be used for controlling populations of *Dendroctonus frontalis* and *D. ponderosae* (Coleoptera: Curculionidae) (Fettig and Munson, 2020), and *Xyleborus glabratus* (Coleoptera: Curculionidae). Some parasitoids, such as *Anagyrus* sp. nov. nr *pseudococci* (Hymenoptera: Encyrtidae) are attracted by the sex pheromones of their target hosts, which act as kairomones for the parasitoids (Franco et al., 2008).

More than 3000 compounds that act as semiochemicals for various insects have been determined. Semiochemicals have been used for pestiferous-arthropod management for more than 100 years. Insect sex pheromones are widely used for monitoring of some species of the Lepidoptera and Coleoptera. The different types of pestiferous arthropods have been successfully managed by employing various semiochemicals (Table 4). Semiochemicals are safe and environmentally friendly because of their natural origin, low persistence in the environment, high species specificity, lack of residues, and safety to non-target organisms. However, there are some difficulties in the practical use of semiochemicals in pestiferous-arthropod management. Pheromone components that either promoted or hindered adoption for pestiferous arthropod management have included biological differences in mate-finding behaviour of different species, complications of chemistries involved, challenges in producing the controlled-release dispensers, and the discovery of effective trap designs. In addition, the political, economic, and use-patterns, particularly in governments regulations of pheromone application make them challenging. In India, the focal pestiferous arthropod species where semiochemicals are playing a major role in IPM programmes are limited. It is largely confined to the Tephritidae through male annihilation technique (MAT) on *Mangifera indica* (Anacardiaceae), species of Cucurbitaceae, and other crops viz., *P. guajava* (Myrtaceae), species of *Citrus* (Rutaceae). No serious efforts have been made about chemo-behavioural strategies of the Tephritidae in India involving host kairomones and male-based

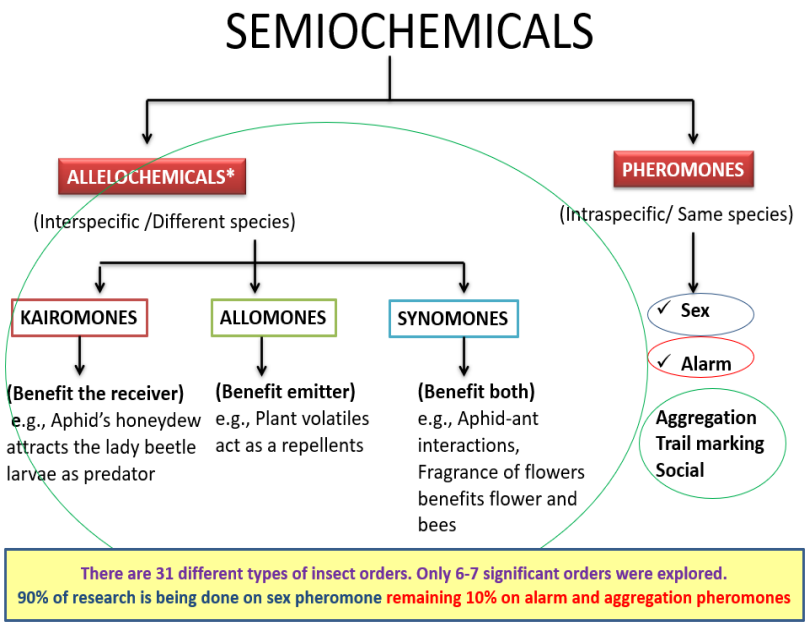

Fig. 2. Semiochemicals in IPM- In agricultural settings, sex, alarm, repellent and aggregation pheromones have been employed for monitoring, mass trapping, male annihilation techniques and auto-confusion. Only few allelochemicals are employed for pestiferous arthropod management, remainder need to improve.*Allelochemicals can be further sub-divided into three groups – kairomones, allomones and synomones (bluish-green circle line).

Table 4. Examples of pestiferous arthropods successfully managed employing various semiochemicals

Pheromones	Compounds	Pestiferous arthropod	References
Aggregation pheromones	$^{+}$neryl(S)-2-methylbutanoate, (R)- lavandulyl acetate, $^{+}$(R)-lavandulyl-3-methyl-3-butenoate	*Frankliniella occidentalis, Thrips palmi* Various Cerambycidae (Coleoptera) *D. ponderosae*	Kirk, 2017
	4,6,6-trimethylbicycle [3.1.1.] hept-3-en-2-one, Verbenone	*Rhynchophorus ferrugineus*	Silva et al., 2017 Fettig and Munson, 2020
	4-methyl-5-nonanol and 2,4-methyl-5-nonanone (9:1), Ethyl-4-methyl octanoate		Chakravarthy et al., 2014
Sex pheromones	dodecan-1-ol acetate, (Z)-7-dodecen-1-ol acetate, 11-dodecen-1-ol acetate, (Z)-9-tetradecenal, (Z)-9-tetradecen-1-ol acetate, (Z)-11-hexadecenal, and (Z)-11-hexadecen-1-ol acetate	*S. frugiperda*	Tumlinson et al., 1986
	(3E, 8Z, 11Z)-tetradecatrienyl acetate	*Tuta absoluta*	Ferrara et al., 2001
Attractants	Methyl eugenol (ME) and raspberry ketone	*Bactrocera dorsalis, B. curcurbitae*	Oliver et al., 2002
Putative sex pheromone	Lignoceryl acetate (24Ac), Lignocerol	*Diaphorina citri*	Zanardi et al., 2018

sex pheromones. Apart from the Tephritidae, the other important pestiferous arthropods affecting fruit trees where semiochemicals can play a key role in strengthening the existing IPM strategies are *Sternochetus mangiferae* (Coleoptera: Curculionidae), *Idioscopus* sp.(Hemiptera: Cicadellidae), *Deonalis albizonalis* (Lepidoptera: Crambidae), *Citripestis eutraphera* (Lepidoptera: Pyralidae), *Procontarinia matteiana* (Diptera: Cecidomyiidae), and *Erosomyia indica* (Diptera: Cecidomyiidae) (Jayanthi et al., 2015). Identifying potential semiochemicals for several Indian horticultural crop pests is still rudimentary.

Semiochemicals are marketed variously, including as pastes, sprays, and baits. Specialized pheromone and lure application technology (SPLAT), which was developed by ISCA technologies, Inc. (Integrated Pest Management Solutions for Sustainable Agriculture) in California, is used for the management of various pestiferous Lepidoptera and Coleoptera, such as *P. gossypiella*, *Anomala orientalis* (Coleoptera: Scarabaeidae), and *D. ponderosae*. In India, various field trials were conducted for *P. gossypiella* using auto-confusion techniques by the Hyderabad-based company ATGC Pvt. Ltd., collaborating with the Junagadh Agricultural University, Junagadh and University of Agricultural Sciences, Raichur. On the other side, SPLAT–Bloom is used to manage pollination by *Apis mellifera* (Hymenoptera: Apidae). Semiochemical technology needs more attention and research in the Indian subcontinent to move beyond this point of adoption. The IPM pheromone market is expected to grow at a CAGR of 12.3% from 2021 to 2028 to reach $1.54 billion by 2028.

Some of the volatile organic compounds emitted by microorganisms (MCOVs) and herbivore induced plant volatiles (HIPVs, stress volatiles) may be useful in managing inter- and intra-specific and tritrophic interactions in future (Aartsma et al., 2017). Microbial VOCs exhibit various biological properties beneficial for plant health, such as enhancing plant growth, inducing resistance against abiotic and biotic stress and inhibiting spore germination and mycelial growth of plant pathogens (Vlassi et al., 2020). Himanen et al. (2017) reported the role of VOCs of *Brassica* sp.in mediating and modifying insect behaviour and their potential in the development of VOC-based crop protection strategies combined with other established methods in the control of pestiferous insects. On the other hand, HIPVs are involved in plant communication with natural enemies of herbivorous

insects, neighbouring plants, and different parts of damaged plant. The release of a wide variety of HIPVs in response to herbivore damage and their role in plant plant, plant herbivorous insect parasitoids, and intraplant communications represents a new facet of the complex interactions among different trophic levels. These volatiles are released from leaves, flowers, and fruits into the atmosphere or into the soil from roots in response to herbivore attack. Moreover, these volatiles act as feeding and oviposition deterrents to insect pests. These volatiles also mediate the interactions between the plants and the microorganisms. An overview of these volatiles emitted by plants, their role in plant defense against herbivores, and their implications for pestiferous insect management need to be exploited more.

CONCLUSIONS

Injudicious use of pesticides leads to a hazardous impact on the environment and humankind. To reduce their negative impact, alternative approaches need to be implemented in IPM and sustainable farming practice. Biologicals and biological controls, microbials and semiochemicals are used as useful and effective alternatives in IPM. However, while implementing these alternatives, farmers face several challenges. To overcome the challenges, adequate knowledge about the new techniques and ecology of pestiferous arthropods will need to be informed to farmers and other business stakeholders. Adequate support for plant protection research is essential to meet the challenges of producing healthy food from the available land with minimal adverse effects on the environment. This can be achieved through the development of a consortium approach involving international organizations, national agricultural research and extension systems, non-governmental agencies, and farmers in the research agenda to meet the needs.

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LIST OF REVIEWERS FOR INDIAN JOURNAL OF ENTOMOLOGY VOLUME 84, 2022

A Kalaisekar, Hyderabad
drkalaisekar@rediffmail.com

Abhishek Shukla, Navsari
abhishekshukla@nau.in

Akash Nikoshe, New Delhi
akashnikoshe1596@gmail.com

Amalendu Ghosh, New Delhi
amal4ento@gmail.com

Amit Choudhary, Ludhiana
amitento@pau.edu

Amutha M, Coimbatore
amuento@yahoo.co.in

Anantanarayanan Raman, Australia
anant@raman.id.au

Ananthi Rachel, Chennai
ananthirachel@mcc.edu.in

Ankita Gupta, Bangalore
ankitagupta.nbaii@gmail.com

Anoop Tiwari, New Delhi
anooptiwariento@gmail.com

Anusha Mohankumar, Bangalore
anushamohankumar0@gmail.com

A P Padmakumari, Hyderabad
padmakumariento@gmail.com

Aparna Kalawate, Pune
aparna_ent@yahoo.co.in

Archana Anokhe, New Delhi
anokhearchana12@gmail.com

Arun Pratap Singh, Dehradun
ranoteaps@gmail.com

Arup Kumar, Jorhat
ak_barbhagia@rediffmail.com

Avinash Chauhan, Medziphema
avi_shimla@hotmail.com

B Ramesha, Kasargod
b.ramesha@gmail.com

Bhushan Ashokrao Thakre, Nagpur
tbhushan50@gmail.com

Barsa Baisalini, Bhubaneshwar
pandabarsa2015@gmail.com

Berin Pathrose, Vellanikkara
berinpathrose@gmail.com

Bhagyashree S N, Shivamogga
bhagyashree.sn@gmail.com

C M Karthik, hivamogga
karthikcmag62@gmail.com

Chinna Babunaik, Nagpur
chinnaenton@gmail.com

Chitra N, Coimbatore
chitra.bookworm@gmail.com

C P Srivastava, Varanasi
csrivastava63@gmail.com

D Sundararaju, Bangalore
rsundariwst@gmail.com

Deeksha Arya, Nainital
deekshu21feb@gmail.com

Devanand Bankar, Latur
devanandbankar@gmail.com

Devinder Kaur, Ludhiana
dkocher2002@pau.edu

Kuldeep Sharma
ujalakuldeep143@gmail.com

Dushyant Raghav, Ramgarh
dushyantiari@gmail.com

Ganesh Behere, Umiam
ganeshbehere@gmail.com

Gopalswamy Sivakumar, Bangalore
sivakumarg.nbaii@gmail.com

Gundappa B, Lucknow
gundu6100@gmail.com

Guru Pirasanna Pandi, Cuttack
guruagri@gmail.com

Harish G, Junagadh
hari4065@gmail.com

Harpal Singh Randhawa, Gurdaspur
harpals_randhawa@pau.edu

Haseena Bhaskar, Thrissur
haseena.bhaskar@kau.in

Babunaik Kalita,
hiakalita23@gmail.com

Jagdish Jaba, Hyderabad
j.jagdish@cgiar.org

Javaji Chandrasekar
javajics@gmail.com

Jharna Chakravorty, Hyderabad
jharna.chakraborty@rgu.ac.in

Joseph Rajkumar, Kayamkulam
joseph.rajkumar@icar.gov.in

Jugal Kishor Bana, Jaipur
jugalbana@gmail.com

K J David, Bengaluru
davidento@gmail.com

K Selvaraj, Bengaluru
selvaentomo@gmail.com

Kailash Naga, Shimla
nagakailash@yahoo.com

Kalleshwara Swamy C. M., Shivamogga
kalleshwaraswamycm@uahs.edu.in

Kamal Ravi Sharma, Varanasi
kamalr.sharma4@bhu.ac.in

Kesavan Subaharan, Bengaluru
subaharan_70@yahoo.com

J Kousika, Coimbatore
kousikajayaram@gmail.com

K V Raghavendra, New Delhi
raghavendrakv70324@gmail.com

Liyaqat Ayoub, J&K
liyaqatayoub@gmail.com

M Ranjith, Shivamogga
ranjithagriofficial@gmail.com

Mahendran,
mahi.iari@gmail.com

Mandeep Pathania, Bathinda
mandeeppathania999@gmail.com

Manishkumar Jayrambhai Joshi, Palampur
mj1107006@gmail.com

Manoj Kumar, Ludhiana
mansnr@pau.edu

Manpreet Kaur Saini, Ludhiana
mksaini@pau.edu

Mohanasundaram, Ranchi
mohaniinrg@gmail.com

Mukul Kumar Deka, Jorhat
mkdeka1968@yahoo.co.in

Nagappa Srinivas, Bengaluru
nagappasrinivas@yahoo.com

Nagesh Chikkarugi, Mandya
chikka68@gmail.com

Naveen Jangir, Hanumangarh
jangir000naveen@gmail.com

Naveenkumar Patil, Cuttuck
patil2850@gmail.com

Nayan Roy
nayan909@gmail.com

Neetu Singh, Noida
nsingh19@amity.edu

Nikhil Trivedi, Anand
nikhiltrivedi00@gmail.com

Nisha Vashishat, Ludhiana
nisha.vashishat@pau.edu

Nitin Kulkarni, Ranchi
Kulkarni_n27@hotmail.com

G S Panduranga, New Delhi
gs.panduranga@angrau.ac.in

P S Amrutha Enkakkad, Wadakkanchery
amruthaenkakkad@gmail.com

Prabhulinga T, Nagpur
prabhut07@gmail.com

Pragna Hittesh Parikh, Vadodara
php59@yahoo.co.in

Pranabesh Nandi, Mohanpur
pranabeshnandi5@gmail.com

Prasanna lakshmi Ravuri, Kalikiri
kvk.kalikiri.smspp@angrau.ac.in

Prasannakumar N. R., Bangalore
prasannakumar.nr@icar.gov.in

Prem Chand Sharma, Shimla
premsharma@hillagric.ac.in

Prerna Sood, Ludhiana
prernasood@pau.edu

Priyanka Bhargava
priyankabhargava019@gmail.com

R Swaminathan, Udaipur
udaiswami57@gmail.com

Rachana Remani, Bangalore
vavarachana@gmail.com

Rachna Pande, Nagpur
rachna.ento@gmail.com

Rachid B
rachid.bouharroud@inra.ma

Rajgopal, Ranchi
raju924rg@gmail.com

Rajwinder Singh, Ludhiana
rajwinder-singh@pau.edu

Rakesh K Gupta, Jammu
rkguptaentoskuast@gmail.com

Rakesh Kumar, New Delhi
rkseth57@gmail.com

Rakesh Pandey, Banda
pandeyent@gmail.com

Reena Sinha, Bari- Brahmna
bkreena12@gmail.com

Roni Kumar Singh, Kolkata
Lucicrohn@gmail.com

Rudra Narayan Borkakoti, Jorhat
rnbk.agri@gmail.com

S Subramanian, New Delhi
entosubra@yahoo.co.in

Sabina Langthasa, Dibarai
sabinalangthasa@gmail.com

Sabu Thomas, Kozhikode
sabukthomas@gmail.com

Sachin Kumar Jaiswal, Raipur
entomologysachin@gmail.com

Sachin Suroshe, New Delhi
sachinsuroshe@gmail.com

Sajad Mir, Kashmir
drsajadento@skuastkashmir.ac.in

Sameer Kumar Singh, Ayodhya
skbhu1991@gmail.com

Samiran Chakrabarti, Kolkata
chakrabarti32b@gmail.com

Sampath Kumar
m.kumar1@icar.gov.in

Samuel Tennison, Chennai
samtennyson@gmail.com

Sandip Patra, Umiam
sandippatra47@gmail.com

Sankararaman
sankararaman05@gmail.com

Santhosh Goud, Ganjam
santhosh1090696@gmail.com

Saranya M, Coimbatore
suganpraveen9@gmail.com

S B Suby, New Delhi
subysb@gmail.com

T Seenivasagan, Gwalior
seenivasagan@yahoo.com

Shakti Kumar Singh, New Delhi
shaktingp@gmail.com

Shalini Santhamma, Bangalore
shalinis.nilavu@gmail.com

M Shankar, Nalgonda
shankar.ento2007@gmail.com

K Shankarganesh, Coimbatore
shankarento@gmail.com

Shankara Murthy M, Bheemarayananagadi
smurthyent@gmail.com

Sharanabasappa, Shivamogga
sharanu.deshmukh@gmail.com

Shashank P R, New Delhi
spathour@gmail.com

Shashikant S Udikere, Dharwad
ssudikeri@gmail.com

Shiddalingappa V. Hugar, Belagavi
hugars2000@gmail.com

Shilpa
shilpa.itgirl@gmail.com

Shravan M Haldhar, Bikaner
haldhar80@gmail.com

Sachin Mahadev Chavan, Panvadi
sachinento@gmail.com

Smriti Sharma, Ludhiana
smritisharma80@pau.edu

Somanath Sahoo, Korapalli
cool.somanath@gmail.com

Ngangbam Somen Singh, Aizawl
somenngangbam87@gmail.com

Sonai Rajan Thangaraj, Coimbatore
snraja_insect@yahoo.co.in

Souvik Sen, Kozhikode
sensouvik07@gmail.com

Subbiah Manikandan, Puducherry
subbiahpoopathi@rediffmail.com

K A Subramanian, Chennai
subbuka.zsi@gmail.com

G K Sujayanand, Kanpur
sujay2020@gmail.com

Sunil Bhalkare, Akola
sunilento@gmail.com

Sunil Joshi, Bengaluru
sunjoshi.pdbc@gmail.com

Suprakash Pal, Cooch Behar
palsento@gmail.com

Surajit Kalita, Jorhat
surjit_kalita@yahoo.com

Suresh Nebapure, New Delhi
smnebapure@gmail.com

Sushil Saxena, Navsari
saxenasushil2003@gmail.com

SVS Gopalaswamy, Guntur
paulgopal2003@yahoo.com

Syamala G
gsyamalaguggilla@gmail.com

Syed Mohammad Ibrahim
syedali.s1966@gmail.com

T Srinivasan, Coimbatore
entosrini@gmail.com

Timmanna Nayaka, New Delhi
thimsento@gmail.com

Ujjwal Kushwaha, Nepal
kushujjwal@gmail.com

Vijay Laxmi Rai, Ayodhya
vijaylaxmi31981@gmail.com

Vikas Tandon, Palampur
tandonvikas8763@gmail.com

Vishlesh Nagrare, Nagpur
vs.nagrare@gmail.com

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