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a) Ventral view



b) Dorsal view

Fig. 1. Male, *Ypthima parasakra* Eliot
For details see page No. 765 of this issue

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STUDIES ON BIOLOGY, MORPHOMETRICS AND SUBSTRATE PREFERENCE OF THE BURROWING COCKROACH *PYCNOSCELUS SURINAMENSIS* (L)

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ABSTRACT

Biology of the burrowing cockroach *Pycnoscelus surinamensis* (L.) revealed that there are four instars and took 134.8± 4.94 days to complete lifecycle. Morphometric studies showed increase in antennal body length, head width, pronotum length and width from first to fourth instar with peak at adult and other developmental stages well fit into Brooks-Dyar rule. All body parameters showed hypoallometry except pronotum length which showed hyperallometry. In substrate preference studies, the survival was lowest for the treatment without substrate and against the treatments with substrates like leaf litter, cocopeat and vermiculite. The mean number of offspring produced and survived was found to be lower in the treatments without substrate and with leaf litter against the treatments with cocopeat and vermiculite.

Key words: *Pycnoscelus surinamensis*, cockroach, instar, hypoallometry, hyperallometry, young ones, fecundity, substrate, leaf litter, life cycle, morphometrics, instar, pronotum, survival, offspring produced

Cockroaches (Blattodea) are among the oldest insects (Appel, 2021) and are nocturnal, typically ground-dwelling insects that evolved around 359.2 million years ago during Carboniferous period. Cockroaches are thought to have first appeared in the Cretaceous period around 100 million years ago (Grimaldi and Engel, 2005). An estimated 4000 species of cockroaches were recorded worldwide, of which, most of the species were in the tropical areas (Roth, 2003). Less than 30 of the 4000 species of cockroaches have been identified as pests around the world (Hedjouli et al., 2021). The evolution of cockroach proceeded in two lines, one leading to the superfamily Blattoidea (families Cryptocercidae and Blattidae), and the other to the superfamily Blaberoidea (families Polyphagidae, Blattellidae, and Blaberidae) (Mckittrick, 1964). About 1020 described species were included in the family Blaberidae, and is the most recently evolved family that has experienced the most extensive adaptive radiation (Siddiqui et al., 2023). The members of Blaberidae are normally found under logs, in humus, compost files, trash, other debris and some are arboreal. A few species, such as *Pycnoscelis surinamensis* (Linn.) are occasionally found to associate with humans and are commonly known as Surinam cockroach. *P. surinamensis* is found in greenhouses, warmer climates and outdoors besides chicken houses and is a known intermediate host for the chicken eyeworm nematode (Gillott, 2005). It is classified as peri-domestic and can only be found near human constrictions or crops. The species reproduces mostly through parthenogenesis and has almost or entirely female populations (Zangl et al., 2019; Rust et al., 1995).

Adults are about 18-25 mm long with dark brown to black bodies and shiny paler brown wings. At birth, nymphs are 4.5 mm long (Cochran and WHO, 1999). There are 15 species in the genus *Pycnoscelus*, some of which lack character visualisation, such as *P. surinamensis*, *P. indicus* and *P. striata* (Bruijning, 1948). Biology of *P. surinamensis* has not been described in India, probably because of its long life (Rau, 1940). A few studies are available elsewhere in India (Cornwell, 1968). Subsequent observations illustrate that the roaches are widespread (Wright, 1973). It takes six months to complete its life cycle and it has not yet been widely researched, especially in the subtropical region of India (Pomes and Schal 2020; Schal et al., 1984). The visualization outcomes shed light on areas that need further investigation. Very little information is available on *P. surinamensis* currently. So, the present study was conducted to observe and disseminate the information regarding biology, morphometrics and substrate preference of this species.

MATERIALS AND METHODS

The study was carried out in the Department of Entomology, University of Agricultural Sciences,

Gandhi Krishi Vigyana Kendra (GKVK), Bangalore (13.07N,77.57E) during 2021-2022. A colony of *P. surinamensis* was collected from College of Sericulture, Chinthamani, Karnataka and maintained in the laboratory at the study site. The colonies were maintained in plastic containers (90 × 60 × 15 cm) and were covered with muslin cloth to prevent cockroaches from getting out and other predators from getting in. Cocopeat was provided as basal substrate which served as shelter and gave the cockroaches a place to hide and breed. Rearing boxes were kept clean and hygiene with attention to clean the bottom of the enclosure at 5-7 days interval. The cockroaches were supplied with vegetable kitchen waste as food and water was sprinkled inside the plastic containers in order to provide sufficient moisture required for the cockroaches. Nymphs were reared inside circular plastic containers (20 cm height and 15 cm diameter) by providing food and water as mentioned above. The mixture of carrot, radish, gliricidia leaves, jackfruit leaves and cashew leaves were provided as food at all the times. Care was taken to keep the diet dry to prevent growth of mould, bacteria, mites, beetles etc. The culture maintained in the laboratory was used for further studies.

Lifecycle was studied by maintaining ten adults separately in a plastic container at room temperature nearly (22ºC), average relative humidity of 64%, by providing sufficient vegetable kitchen waste as feed. This species reproduces by ovoviviparity. Adults were inspected regularly and the data regarding young ones laid was recorded on daily basis. Observations such as total number of young ones laid by each female (fecundity of female), days taken to first moult and subsequent moults, duration of each instar, total number of days taken to complete the life cycle and adult longevity were recorded in the data sheets. Morphometrics were carried out using Leica stereozoom microscope (Leica MZ 6, Solms, Germany). 25 nymphs of each instar were collected and was anesthesized using 95% ethanol and measurements were taken. Adults were subjected to freezing in the refrigerator for ten minutes for the studies, as alcohol caused the adults to shrink, making it difficult to measure. The measurements recorded were body length (anterior tip of the head to the tip of the abdomen in nymph and from front of the head to the tip of the wings in adults), antennae length, head width (between the outside edges of the eyes), pronotum length (along the dorsal midline) and width (at its widest point), intraocular distance and forefemur length in mm. The dispersion of the mean values of all biological parameters and body measurements were worked out by calculating standard error of mean (SEm).

The substrate preference of *P. surinamensis* was studied using four treatments and five replications, where ten adults were used per replication making 50 adults per treatment. A circular plastic container (18 cm height, 12.5 cm dia) was used to study the substrate preference of *P. surinamensis* and ten adults were released per replication and was provided with fresh vegetable kitchen waste as food for all the treatments. In the treatment one, the adults were maintained in the container without substrate. In the treatment two, the adults were released into plastic container filled with leaf litter as a substrate for about 4 cm depth and were allowed to lay young ones. In the treatment three, the plastic containers were filled with cocopeat of about 4 cm depth and in the treatment four, vermiculite of about 4 cm depth were filled and adults were released and allowed them to feed and lay young ones. The preference of *P. surinamensis* for suitable substrate was determined by taking the observations at weekly intervals such as survival percent of adults and the growth of young ones.

RESULTS AND DISCUSSION

Biological studies on *P. surinamensis* revealed that there were four instars in the nymphal stage. The mean developmental duration of first, second, third and fourth instar were observed to be 30.5± 1.55, 31.5± 1.55, 34.25± 2.63, 29± 1.08 days and the adult longevity was 111.25± 3.35 days, respectively (Table 1) and the total number of days taken to complete the life cycle was 134.8± 4.94 days. In Turkestan cockroach male and female nymphs developed into adults on an average of 222 and 224 days, respectively (Kim and Rust, 2013). Similar studies in American cockroach, *Periplaneta americana* (L.) revealed that it takes about 160.5 days to 25 months to develop from hatching to the adult stage. The incubation period was found to be 35 days and the interval between the brood varies from 48-82 days (Borah and Hazarika, 2019). Similar type of studies reported an incubation period of 24-38 days with *P. americana* (Perrot and Miller, 2010). The female produces 1-5 broods (3 average). Adult females deposited up to 25 oothecae. The oothecae averaged 16.8 eggs and 13.9 nymphs emerged/ egg capsule, resulting in 82.7% hatching rate. The results are in accordance with those on the egg cases of cockroach that these contained 16-50 eggs (Hahn and Ascerno, 2005). Presence of 12-40 eggs in ootheca has been reported in *P. americana* (Whitworth and Ahmad, 2007). It was observed that the antennal length, head width, body length, pronotum length and pronotum width increased from first to fourth instar and peaked

Table 1. Morphometrics and growth ratios of *P. surinamensis* (in mm) (n=25)

Instar	Duration	Antennal length (Mean± SEm)	Head width (Mean± SEm)	Body length (Mean± SEm)	Pronotum length (Mean± SEm)	Pronotum width (Mean± SEm)	Intraocular distance (Mean± SEm)	Forefemur length (Mean± SEm)
Morphometrics								
I	30.50±2.18	2.24± 0.07	1.41± 0.06	5.77± 0.23	0.85± 0.03	2.69± 0.04	0.81± 0.02	0.74± 0.01
II	31.50±1.55	3.49± 0.11	1.97± 0.06	10.36± 0.26	1.45± 0.1	3.81± 0.04	1.37± 0.01	1.26± 0.02
III	34.25± 2.63	5.35± 0.15	2.53± 0.02	15.04± 0.25	2.63± 0.04	6.38± 0.06	1.71± 0.04	1.76± 0.02
IV	29.00± 1.08	6.35± 0.12	2.91± 0.06	18.67± 0.34	3.76± 0.12	7.55± 0.04	1.90± 0.02	2.04± 0.02
Adult	111.25± 3.35	7.12± 0.19	2.85± 0.08	22.17± 0.57	4.31± 0.12	7.68± 0.11	1.99± 0.06	2.28± 0.02
Growth ratios								
Growth ratios		Antennal length (in mm)	Head width (in mm)	Body length (in mm)	Pronotum length (in mm)	Pronotum width (in mm)	Intraocular distance (in mm)	Forefemur length (in mm)
II/I		1.558	1.397	1.795	1.706	1.416	1.691	1.703
III/II		1.533	1.284	1.452	1.745	1.675	1.248	1.397
IV/III		1.187	1.150	1.241	1.486	1.183	1.111	1.159
Adult/IV		1.121	0.979	1.187	1.146	1.017	1.047	1.118

at the adult stage (Fig. 1). All the body parameters like antennal length, head width, body length, pronotum width and fore femur length showed hypoallometry while the pronotum length showed hyperallometry (Fig. 2). The present findings agree with the fact that a series of allometric changes occurred at morphological level during the development of an insect (Borah and Hazarika, 2019). Observations from the morphometric studies also revealed that, the body measurements of the nymphs and adult was found to be significantly larger than the preceding instar in all cases except the pronotal length between the fourth instar and adult stage. In invasive Turkestan cockroach, *Blatta lateralis* they observed that in all cases except the pronotal length between the fourth and fifth instars, each instar was significantly larger than the preceding instar (Lee et al., 2021). According to the Brooks-Dyar rule (1890): median growth ratio for paurometabolous insects varies from 1.4 to 1.7 and this equation that, postmoult size divided by premoult size is a constant.

In the analysis of the present study, the growth ratios of body parameters were calculated, although the results of analyses support the Brooks-Dyar rule, they do not follow the rule and the values of ratios were decreasing from the first instar to adult and they were not constant. Similar to this in *Blaptica dubia* there were seven instars and the growth ratio followed the Brooks-Dyar rule (Wu H, 2013). He observed the growth ratio of pronotal length, pronotal width and head width were 1.26, 1.24, and 1.19, respectively. *B. dubia* shared a similar growth pattern with other paurometabolous insects (Wang et al., 2021).

Data on the effect of substrate on the survival and number of offspring produced revealed that among the four treatments, the treatment (T_1) without substrate varied significantly and were able to survive only up to eleven weeks. There was no significant difference with respect to survival and offspring production between leaf litter, cocopeat and vermiculite substrates whose survival was highest up to 16 weeks. The mean number of offspring laid in different treatments was observed. Among the four treatments, the treatment (T_1) without substrate and the treatment (T_2) with leaf litter has lower mean values of young ones compared to the treatment (T_3) cocopeat and the treatment (T_4) vermiculite, which had significantly higher mean values of young ones. The findings of substrate preference studies were similar with the investigations that reported highest mature weight, survival and overall performance index depended on the substrate on which the cockroach

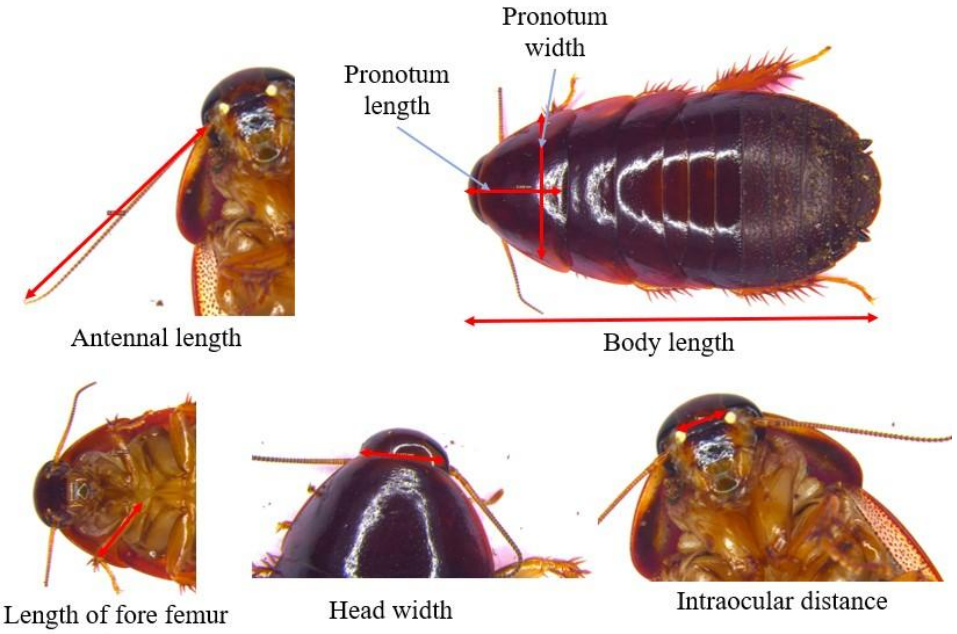


Fig. 1. Morphometrics of *P. surinamensis*

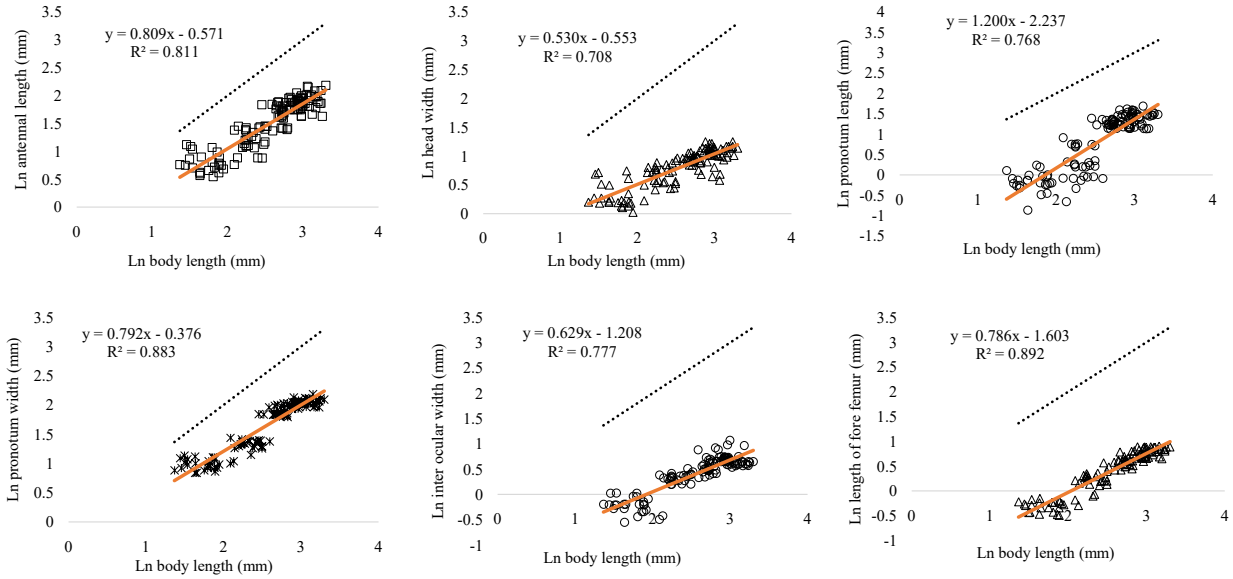


Fig. 2. Ontogenetic scaling of body parts measured through the developmental stages in *P. surinamensis.* (Values plotted on a log-log scale using natural logarithms)

was maintained (Ngaira et al., 2022). The outcome was similar with the findings that had investigated the site preference and oviposition behaviour for oothecal deposition by *Supella longipalpa* (F.) females (Benson and Huber,1989). They stated that, the behavioural sequence and oviposition site preference were determined by type of substrate on which the insects were maintained (Miller and Smith, 2020; Smith et al., 1999). Leaf litter, cocopeat and vermiculite have the ability to store moisture and thus influenced the stability of temperature (Weinstein, 1994). More studies would reveal the ideal condition of temperature and humidity for better survival of these roaches. Lack of any substrate resulted in dry and wet conditions coupled with fluctuations in temperature condition (Rahimian et al., 2019). Thus, humidity and temperature factor may have significant impact on the survival and growth of *P. surinamensis*.

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AUTHOR CONTRIBUTION STATEMENT

Sumithramma N conceptualized and framed the research proposal, Gowrisankar Reddi conducted the experiment, curated data and prepared original draft. Vidya Mulimani, Sanjay Kumar Pradhan, A. R. V. Kumar contributed to the samples, analyzed the results and corrected draft. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF TEMPERATURE ON THE COMPETITIVENESS OF THREE STORED PRODUCT INSECTS

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ABSTRACT

Competitiveness is an important feature in insect biology. Three srtored product pests viz., *Trogoderma granarium, Tribolium castaneum, Oryzaephilus surinamensis* were evaluated for their competitiveness at 35, 25 and 40 °C. The results revealed that within 6 months, *T. castaneum* and *O. surinamensis* could not displace the *T. granarium*, but at 35 °C, *T. granarium* was able to displace the *O. surinamensis*. It was found that *T. granarium* at 25 °C continued to be dominant for long periods without being affected by other species. It was in the dormant larval or egg stage despite the death of all adults, and the increased at 40°C.

Key words: *Trogoderma granarium, Tribolium castaneum, Oryzaephilus surinamensis*, superior, competitor, density, heat, determinants, competition, tolerance, preferences, dormancy, displacement, abundance

Competition theory had an important role in the development of general environmental theory and the interpretation of data. Competition is one of the basic vital factors that shape patterns of distribution, increase, and diversity in ecological communities (Tikhonov and Monasson, 2017), Invasive species are thought to be highly competitive, but few have failed to prove themselves. It turns out that competitiveness is related to the biotic resistance (Yannelli et al., 2017). *Trogoderma granarium* larvae have a long dormant period. *Trogoderma granarium, Tribolium castanum*, and *Tribolium confusum*, the number of eggs laid by females was affected by the relative humidity; and *T. castaneum* and *T. confusum* could not lay eggs 20 and 30% RH. Highest percentage of eggs hatched at 50% RH 20% RH prevented the larvae from turning into pupa (Falah ad Azher, 2020). The saw-grain beetle, *Oryzaephilus surinamensis* is an important pest of stored food (May, 2022). Competition among insects increases with the increase with population density and physical closeness. Evolution and competition tend to be stronger between insect food specialists in so far as they affect general feeding. However, specialized overlap is not everything (Bird et al., 2019).

Despite studies on competition behaviour in beetles of stored products, many have focused on competition for one species. This competition is determined by many biotic and abiotic conditions, including temperature and type of food. There is still insufficient information on the effect of these conditions on the competition of *T. granarium* with other insects (Guedes et al., 2010; Oliveira et al., 2015; Kavallieratos, et al., 2017). The present study aims to evaluate the effect of temperature on the the competition between three stored products pests viz., *T. granarium, T. castaneum* and *O. surinamensis*.

MATERIALS AND METHODS

The three species of beetles *T. granarium, T. castaneum* and *O. surinamensis* were obtained from the Store Pests Laboratory, Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad. The mass culture was raised on crushed wheat, to detect the dominant species. Three competition experiments were conducted for each two species separately, at three temperatures (25, 35, and 40 °C), and for three storage periods (60, 120, and 180) days. Each treatment was replicated thrice, and each replicate included 20 pairs of adults (Qader et al., 2022). SAS (2018) program was used to evaluate their significance using ANOVA (LSD, p=0.05) was used.

RESULTS AND DISCUSSION

Competition between two types of storage insects and at a temperature of 25 °C and a relative humidity of 65% and periods of 60, 120, 180 days, when evaluated maximum population density (245.67 individuals) for the *T. castaneum*, when competing with the *O. surinammsis*. These environmental conditions are best for *T. castaneum*, but worse for *T. granarium*, whose

larvae have entered a dormant state, or their adults are killed, which did not allow an increase in their population density. The statistical analysis indicated that the results were significant for the change in population density, as well as for the overlap between time and the change in population density. Table 1 reveals the population density and competition between two species at 35 °C and 65% relative humidity at 60, 120 and 180 days, respectively. These data reveal maximum population density @396.67 individuals for *T. castaneum*, when it competed with the *O. surinamensis* @ (30.00 individuals). It is followed by population density @345.33 individuals for *T. granarium*, when it competed with *O. surinamensis*, @ 24.33 individuals. It is followed by the third level of population density, @ 311.66 individuals for *T. castaneum*, when it competed with *T. granarium*, (@139.00 individuals). These environmental conditions show the best for *T. castaneum* and the worst for *O. surinamemsis*. The statistical analysis indicated that the results were significant for the change in population density as well the overlap between time and change in population density. Table 1 reveals that there was a change in the population density when competing between two species and at 40 °C and a relative humidity of 65% in the periods of 60, 120 and 180 days, respectively. Maximum population density was of *T. granarium* (434.3), when it competed with *O. surinamemsis* (12.33 individuals). It is followed by *T. granarium* (383.67) when it competed with *T. castaneum* (98.33 individuals). It was found that these environmental conditions were the best for *T. granarium*, and the worst for *O. surinamemsis*. Statistical analysis indicated the significance of the results, the change in population density and the progression of time, as well as the overlap between them. Thus, the rise in temperature was the most suitable for *T. granarium*, and in all competitions with others temperature of 25 and 30°C, were the most suitable for *T. castaneum*. There are significant differences in the population density of insects at different temperatures and time periods.

Table 1. Two Species of stored insects competed at 25°C;
Two types of stored insects competed at 35°C; Two types of stored insects competed at 40.0°C

Species	Time/ days			Mean	Time/ days			Mean	Time/days			Mean
	60	120	180		60	120	180		60	120	180	
	Population density				Population density				Population density			
T. granarium	27	19	12	19.33	177	149	91	139.00	231	400	520.0	383.67
T. castaneum	129	197	261	195.67	272	322	341	311.66	93	132	70.0	98.33
T. castaneum	149	276	312	245.67	293	412	485	396.67	63	183	217	154.33
O. surinamemsis	53	42	18	37.67	47	31	12	30.00	33	0.0	0.0	11.00
O. surinamemsis	192	94	88	124.67	51	22	0.0	24.33	37	0.0	0.0	12.33
T. granarium	62	47	28	45.67	286	351	399	345.33	337	421	545	434.33
Mean	102.00	112.50	119.83	---	187.67	214.50	221.33	---	132.33	189.33	225.33	---

LSD value for time: 29.53 NS, population density: 29.53*, overlap: 52.79*; LSD value for time: 36.97 NS, population density: 44.05*, overlap: 71.24*; LSD value for time: 43.91*, population density: 56.07*, overlap: 78.33*

These data also revealed that an increase in the storage period does not necessarily lead to an increase in population density. The environmental impact on insects when they compete, it becomes clear that the difference in the population density occurs as a result of the positive or negative effect of temperature and for different periods (Okrikata and Ogunwolu, 2019). A study of the biology of *T. granarium* described its survival as an invasive species, finding that temperature seriously affects population growth. Knowing the insect's potential growth rate also makes it easier to estimate its numbers over time, and therefore its potential outbreaks. at 40°C, the intrinsic rate of increase indicates that in this temperature range, *T. granariun* is able to increase its population size, as well as its ability to spread, becoming more virulent in stored products (Papanikolaou, et al., 2019). The results present study corporates with those of Collins et al. (2019) when analyzing the stability of competing insect species on a single food source dertemine which species win. Present results are also consistent with those of Lee et al. (2020)

in which synchronization between species is driven by the environment, while competition between species becomes less positive or more negative. In the same way, the strength of competition has a greater negative effect on the relationship between species when one has a more variable growth rate than the other (Lee, et al., 2020). The type of competitor superior in temperature resistance is superior to other competitors (Bale et al., 2002; Thierry et al., 2019). Non-diapausing larvae were more susceptible than diapausing larvae and showed a delayed response in terms of completing their biology to reach the adult stage, in contrast, diapausing larvae had no delayed response compared to the controls, the life stage with the highest level of tolerance in the eggs (Gourgouta and Athanassiou, 2021). An important component of the biology of *T. granarium* is the long diapause in the larval stage (Athanassiou, 2022). In the study under 25± 1 ºC and 50± 5% RH, *T. castaneum* shows the fastest total development (Đukić et al., 2022).

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AUTHOR CONTRIBUTION STATEMENT

The idea of research, conducting experiments, analyzing the results, and writing was done by the researcher.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF PHEROMONE BLEND SOURCES AND LOADINGS ON SUGARCANE INTERNODE BORER MOTH CATCHES IN SRI LANKA

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ABSTRACT

The internode borer, *Chilo sacchariphagus indicus* Kapur is an important moth borer on sugarcane in South Asia including India and Sri Lanka. Collaborative studies to evolve locally optimum sex pheromone trapping system for this pest were undertaken in Sri Lanka during 2019-20. The first study comparing alternative pheromone blend sources showed significant differences in moth catches, with the best source consistently attracting twice or more moths than the others. The next study on pheromone lure loadings showed non-significant differences in moth catches, clarifying the adequacy of the widely adopted loading (3 mg/ septa). While these findings could guide in adopting locally optimum blend source and loading for pheromone trapping of the borer moths, the scope of future R&D to further enhance the impact potential of pheromone-based mass trapping of sugarcane internode borer moths in Sri Lanka is indicated.

Key words: Pheromone trap, sugarcane, *Chilo sacchariphagus,* internode borer, moth borer, sex pheromone, blend source, lure loading, insect sex attractant, IPM, Sri Lanka

Sugarcane is known to suffer significant loss in cane yield and sugar recovery due to the internode borer (INB), *Chilo sacchariphagus indicus* (Kapur) (Lepidoptera: Pyralidae) in India (Sithanantham, 2006) and in Sri Lanka (Wanasinghe et al., 2019). While past research on INB in Sri Lanka has covered action thresholds for local control decisions (Senavirathne et al., 2001), locally available chemical insecticide use options (Kumarasinghe, 2008), and the potential for using borer tolerant genotypes (Fernando et al., 2004), the scope for pheromone-based trapping has not yet been examined locally. In India, the two major sex pheromone components emitted by females of internode borer had been identified as- Z-13-18Ac and Z-13-18OH and their synthetic blend in 7:1 ratio was found to be superior for attracting native males in India (David et al., 1985), while the scope for sex pheromone-based trapping for monitoring and mass trapping of this borer has been well documented (Mukunthan et al., 2003). The presently reported collaborative studies are on relative attractiveness of sex pheromone blend sources/ loadings available from India as tools for enhanced trapping efficiency for INB moths in Sri Lanka whose results are summarised herein.

MATERIALS AND METHODS

The field studies were taken up in Sri Lanka in sugarcane fields at Siyambalanduwan Ethimale and Hingurana during October-November, 2019. For each location about 1 hectare area crop of the same age (4 months) and the variety- SL 96 128 was chosen. The crop was grown as per recommended crop management practices, but without application of insecticides so to ensure adequate INB infestation for the evaluations.

Study 1. Pheromone blend sources testing: The synthetic pheromone of *C. sacchariphagus indicus* (consisting of Z,13-octadecenyl acetate and Z,13-octadecenol) available in 7:1 blend ratio from three blend sources in India (A,B,C) were secured through collaboration with Sun Agro Biotech Research Centre (SABRC), Chennai. All three sources were dispensed at 3 mg loading/ rubber septa lure, and compared between common septa source (a) versus individual commercial source (b) for each. The following six treatments were compared for field attractancy: T1. Source-A.a (common septa); T2. Source-A.b (individual septa); T3. Source-B.a (common septa); T4.Source-B.b (individual septa); T5. Source-C.a (common septa); and T6. Source-C.b (individual septa).

Study 2. Pheromone loadings testing: The pheromone blend with 7:1 ratio was compared in six different loadings (mg/ septa), using common rubber septa dispensers and dispensing method. The following six

loadings were compared: T1. Loading-3 mg/ septa; T2. Loading-5 mg/ septa; T3. Loading-6 mg/ septa; T4. Loading-7 mg/ septa; T5. Loading-8 mg/ septa; and T6. Loading-10 mg/ septa.

In both the locations, the comparison treatments were kept in delta traps and placed at 20 m inter-distance between them in each of four replications. The treatment positions in each replication were assigned at random initially and also interchanged at random within the replication after each weekly count of moths to minimise position effect during the total observation period of four weeks. The moth catch data were subjected to ANOVA as per Gomez and Gomez (1968).

RESULTS AND DISCUSSION

The results of the two field studies are summarised as follows:

Selecting more attractive pheromone blend source: The results are summarised in Table 1. The treatment differences in moth catches were highly significant, also differing between weeks significantly, while interaction between treatments and weeks was minimally significant. The week-wise pattern of relative moth catches showed that the more promising blend (T1) source (A.a) tended to be consistently superior (Table 1, 2). The mean moth catch in this treatment was nearly twice that of next ranking source (C), while being about four times than the other treatments compared.

Table 1. ANOVA results from two locations (pooled data)

Factors	Significance	C.D	F value
Treatments (6)	**	1.53	7.47
Weeks (4)	**	1.25	14.12
Treatments x weeks	*	3.06	2.47

**= Significant at p=0.01; *= Significant at p=0.05; C D=Critical difference (p=0.05)

Table 2. INB Moth catches for six pheromone sources (pooled data)

Treatments (blend source vs. lure type)	Week 1	Week 2	Week 3	Week 4	Mean
T1 (A.a)	10.60	4.00	3.40	0.20	4.55
T2 (A.b)	1.80	0.40	0.40	0.40	0.75
T3 (B.a)	2.60	0.80	1.00	0.60	1.25
T4 (B.b)	0.00	1.40	1.20	0.00	0.65
T5 (C.a)	4.40	2.80	3.40	0.20	2.70
T6 (C.b)	6.40	1.60	2.00	0.00	2.50
Overall mean	4.30	1.83	1.90	0.23	2.07

Blend Source: 1-A; 2-B; 3-C; Lure type-a: common source; b: individual source

Comparing different pheromone loadings in lure: The study on six loadings in the lure showed non-significant differences. The mean catch/ week was 2.55/ trap, and the respective mean weekly catches for the six loadings (3,5,6,7,8 and 10 mg/ lure) were 2.25, 2.58, 2.28, 2.10, 2.30 and 3.78. While moth catch differed significantly between weeks, the interaction between treatments and weeks was insignificant, indicating that the overall trend of relative ranking among the treatments did not seem to vary across the weeks. These data confirm the consistency in lack of significant differences in catches between the loadings tested. Thus, widely adopted loading (3 mg) appeared adequate.

The main outcome from this study is the identification of a more promising pheromone blend (Z-13-18Ac: Z-13-18OH) source (T1) which caught significantly and consistently more male moths among the alternative blend sources availed from India. This result has created a critical knowledge base in Sri Lanka on promising pheromone blend source to attract male moths of *C. sacchariphagus* in the field, and so could be deployed for monitoring or mass trapping of the borer populations in Sri Lanka. This result is comparable in utility to the earlier field attractancy studies in India on the same two female pheromone components (Z-13-18Ac: Z-13-18OH) in a blend ratio of 7:1 by David et al. (1985). The present findings are also in agreement with the findings in Mauritius populations of *C. sacchariphagus* (Nesbitt et al., 1980) wherein the same blend in 7:1 ratio was superior in field attraction to males and comparable in attractancy to the native virgin females.

The apparent consistent superiority of the selected pheromone blend source (A.a) observed from the present four week duration of study, along with the benefit from dispenser choice could guide in availing more efficient blend-dispenser combination for future use locally. The utility of such testing of alternative commercial blends for local optimization of pheromone trap catches has been well documented for the globally invasive pest the fallarmy worm *Spodoptera frugiperda* (J E Smith), wherein strain differences in blend ratios are known (Andradea et al., 2000; Malo et al., 2001).

Further, the present results on loadings indicate lack of significant differences between loadings tested. It could be recommended that the currently widely adopted loading of 3 mg could be used for season-long trapping of this borer in Sri Lanka. These results are

comparable to lure dispenser comparison studies for this borer by Manikandan et al. (2014), as basis for their cost-competitiveness. The present findings also provide a basis to promote pheromone-based trapping for local monitoring of the borer in Sri Lanka, which is already recommended elsewhere by Way et al. (2003) as a low cost option due to ease of operation, and sensitivity at low population levels. This is being already adopted for monitoring the population dynamics of this borer in India (Chatterjee et al., 2007).

Further R&D on pheromone-based mass trapping (Beevoor et al., 1990) and integration with *Trichogramma*-based biocontrol (Bhavani et al., 2016) could help evolving more holistic pheromone-based trapping technology for this borer, as proposed earlier for sugarcane borers in India by Mukunthan et al. (2003).

The present findings of identifying the more promising pheromone blend source from India and the adequacy of 3 mg loading/ lure can provide an interim basis for local pheromone trapping needs in Sri Lanka. There is scope for further collaborative R&D on intraspecies diversity of pheromone blend ratio pattern of native female populations and the response scenario of local male populations to alternative blend sources/ ratios/ loadings in major agroecologies of sugarcane cultivation towards more robust pheromone-based trapping technology as key components for biointensive IPM for this borer pest of sugarcane in Sri Lanka.

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VIRULENCE OF NATIVE ISOLATES OF ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF WHITE GRUBS

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ABSTRACT

An alternative to chemical control, entomopathogenic nematodes are very effective against insect pests. Native isolates of *Steinernema siamkayai*, *S. carpocapsae*, *Heterorhabditis indica*, *H. bacteriophora*, *Heterorhabditis* sp. and *Oscheius* sp. have been evaluated for their virulence against white grubs. Under laboratory conditions, *S. glaseri* was more susceptible to *A. communis* in potato. *S. glaseri* @ 2.5 x10^9 IJ/ ha showed the highest grub mortality of 83.33% and 19.04% tuber damage. The same dosage of *Heterorhabditis* sp. in pot culture caused 18.66% tuber damage and 74.05% larval mortality. *Steirnernema glaseri* and *Heterorhabditis* sp. effectiveness against the coleopteran insect pests of *Anomala communis* in potato was conducted under field conditions. The maximum grub mortality with *S. glaseri* at 2.5 x10^9 IJ/ ha was 71.33%. With *Heterorhabditis* sp. at 2.5 x10^9 IJ/ ha, the yield increased by 14.14 t/ ha while tuber damage was 24.99%. However, the nematode *S. glaseri* and *Heterorhabditis* sp. has its best ability to live at the lowest temperature in the field trial, *Anomala communis* was successfully managed by *S. glaseri* followed *Heterorhabditis* sp. in potato. The natural distribution of the native isolates of EPNs may be used for local insect pest management.

Key words: Native isolates, *S. siamkayai*, *S. carpocapsae*, *H. indica*, *S. glaseri*, *A. communis*, potato, grub morality, tuber damage.

Steinernematidae and Heterorhabditidae entomopathogenic nematodes are the most successful biological control agents against a variety of insect pests (Kaya and Gaugler, 1993). Insect mortality was caused by entomopathogenic nematodes (EPNs) at 48 to 72 hrs. It is an integrated pest management (IPM) approach, the insect killing capacity of EPN is therefore an excellent alternative for chemicals in the management of insect pests. Entomopathogenic nematodes are environmentally friendly and a promising biocontrol agent because of a variety of factors (Ahmad et al., 2009).

The world's most important food crop is the potato (*Solanum tuberosum*). Laznik and Trdan, 2015 recorded the *H. bacteriophora*, used for the control of mixed populations of Scarabaeidae species. In India, white grubs are the most widespread and destructive insect pest. White grubs are a significant category of insect pests that harm to potatoes and are more significant than other grub species (Chandel et al., 2015). The form and colour of all white grub species are the same (Mehta et al., 2010). White grubs consume underground stems and root tubers for food (Veeresh and Rajagopal, 1983). The second and third instars of the young grubs eat tubers (Mehta et al., 2010). The second instar white grubs produce smaller holes in tubers and third instar make large, shallow and irregular cavities into potatoes (Chandel et al., 2003). In India's higher and upper hills, *Anomala communis* has gradually increased its range (Regupathy et al., 1997). In Tamil Nadu key potato-growing regions is Ooty, a hilly state in North West India. Potato tubers with wide, shallow, round holes are destroyed by grubs in their second and third instars (Misra, 1995). The tubers are harmed by grubs, the foliage shows no symptoms. So the tubers low market value was caused by the white grub infection. Since these nematodes live in soil, controlling various soil pests through application in soil is successful. Imidacloprid combined with *Steinernema kushidai* is particularly successful for the control of white grubs, according to Koppenhofer et al., 2000. It is widely accepted that entomopathogenic nematodes can be used as biological control agents in protected habitats. When nematodes infect insect hosts, they release species-specific symbiotic bacteria into the hemolymph of the host, where only infected juveniles can infect the insect host (Kaya and Gaugler, 1993). Our study's objectives include determining which species of entomopathogenic nematodes are the most effective in relation to temperature and nematode concentration. The need of the hour is the possible effectiveness of

EPN in favour of white grub for replacing insecticides with biological control agents. The most successful nematode found in the current field experiment may be used in a long-term strategy to boost potato production. EPNs have been shown to be effective biological control agents of *A. communis* in potatoes when used in high concentrations and in combination with favourable abiotic environmental conditions (high humidity, optimum temperature).

MATERIALS AND METHODS

The native isolates of entomopathogenic nematodes viz., *H. bacteriophora, Heterorhabditis* sp*., H. indica*, *S. siamkayai*, *S. carpocapsae* and *S. glaseri* were isolated from horticulture ecosystem of Tamil Nadu and nematodes culture were mass cultured with *C. cephlonica*. *C. cephalonica* eggs were obtained from the biocontrol laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. Rice meal moth *Corcyra cephalonica* Staint. (Pyralidae: Lepidoptera) was reared on bajra (*Pennisetum typhoides* L**.**) grains. Bajra grains (2½ kg) were broken and mixed with groundnut grains and filled in 30 cm x 20 cm plastic trays and eggs of *C. cephalonica* (1 cc/ tray) inoculated into the trays and covered with a muslin cloth. Fully grown larvae of *C. cephalonica* were collected after 30 days of inoculation and used for further studies.

The entomopathogenic nematodes were continuously sub-cultured on larva of rice meal moth, *C. cephalonica*, which were reared on broken bajra grains in plastic basins. The insect larvae were exposed to the nematode as per the method described by Bedding (1984). About ten final-instar larvae of *C. cephalonica* were released into a Petri dish over two Whatman No.1 filter papers inoculated with infective juveniles stored in sterile distilled water at the rate of 20 per larva (1 ml suspension containing 200 infective juveniles). The Petri dishes were sealed with cling film and stored in polythene bags to conserve moisture. Nematodes were extracted from the cadavers five days later using a White's trap (White, 1927) for *H. indica* and by modified method using plaster of paris (Woodring and Kaya, 1988) for *S. glaseri*, after three days. Nematodes were recovered from the traps daily until exit of infective juveniles ceased. These juveniles were washed and rinsed several times with sterile distilled water and stored in a BOD incubator at 20±1° C for *H. indica* and in a refrigerator at 10°C for *S. glaseri* in 500ml conical flask until use. The nematode cultures were aerated and changed to fresh sterile distilled water at weekly intervals. Entomopathogenic nematodes were selected for testing virulence against 2nd and 3rd instar larvae of *A. communis*. Dose - mortality relationship and time mortality tests were conducted in 9 cm diameter petri dishes lined at the bottom with a Whatman No. 1 filter paper and moistened with 1ml sterile distilled water. Infective juveniles were evenly applied over the filter paper. The dosages used were 0, 50, 100, 200, 400, 800 and 1000 infective juveniles per larva, with 10 larvae per insect per replicate and three replicates for each level. Talc based formulation of EPN powder was autoclaved at 20 lbsp.si. It obtained sterile water at various moisture percentages, including 10, 20, 30, 40, and 50%. It was then injected with 1000 infectious juveniles of virulent strains of entomopathogenic nematodes per kilogram of talc.They were stored in zip-lock polythene covers and incubated in a BOD incubator at a temperature of 20° C. One gram sample of the talc formulation was dissolved in water on a weekly basis, and the number of nematodes that survived was determined. 20 infectious juveniles per larva and 10 *C. cephalonica* larvae per replicate were used to inoculate the infected juveniles onto filter paper. Each nematode underwent four replications and the percent infectivity was calculated. To check the infectivity of entomopathogenic nematodes.

Under greenhouse conditions, pot culture experiments were carried out to evaluate the virulence of entomopathogenic nematodes against 2nd and 3rd instar larvae of *A. communis* on potato. The surface of potato tubers (var: Kufri Jyoti) was disinfected, then they were water washed. Three potato tubers were planted in each of the five kilogramme capacity earthen pots when the tubers were planted. To increase the host suitability of the *A. communis* larvae, after germination and establishment of the seedling, they were infected and fasted for one to two weeks. The nematode treatments contained *S. siamkayai*, *S. glaseri, S. carpocapsae*, *H. indica*, *H. bacteriophora*, *Heterorhabditis* sp, and *Oscheius* sp. at $5x10^9$ IJs/ ha, respectively. Three times each treatment was repeated using a completely random design (CRD). For each treatment, the nematode formulations were applied into the soil. After 96 hours after treatment, insect death counts were recorded every 48 hours. The quantity of dead larvae was counted and nematodes were found inside the bodies. In all of the treatments, damaged tubers brought on by the larvae were also noted.

The effectiveness of native isolates of entomopathogenic nematodes, including *S. siamkayai*,

S. glaseri, S. carpocapsae, *H. indica*, *H. bacteriophora*, *Heterorhabditis* sp. and *Oscheius* sp. against the larvae of *A. communis* was tested in the field conditions. The experiment was carried out in a potato field that was naturally infested with white grubs. There was no population of entomopathogenic nematodes found in the experimental field. A field experiment using the Randomized Blocks Design with three replications was carried out at the farmers field, Ooty. The plants were grown in a plot measuring 12 m^2. In each plot, a random potato (var: Kufri Jyothi) exhibiting signs of damage caused by the pests third and fourth instar larvae was chosen, labelled and the grub population was noted. *Heterorhabditis* sp. and *Steinernema glaseri* at 2.5 x10^9 IJ/ha were used as the nematode treatments. For each treatment, different talc doses/ m^2 of nematodes was inoculated close to the base of potato plants. Distilled water was used to thoroughly wet the control plots.

Grub mortality counts were performed up to 7 days after application at 3-day intervals. The observation was taken at grubs and other life stages were collected from random locations to determine if they were alive or dead and were dissected to determine the presence of entomopathogenic nematodes. Data were collected on grub population/ plot, plant damage/plot and damaged tuber/ plot at harvest. The number of dead grubs were counted and confirmed for the presence of nematodes inside the cadavers. Damaged tubers due to the larvae were also recorded in all the treatments. Bedding and Akhurst's reported that nematode-baiting method was used, utilizing alternative hosts insects as baits (1975). To assess whether the applied EPNs had established and persisted in the field soil. Soil sample were taken from the EPN applied field for white grub assessments. To study the nematode persistence after 90 days post application. The EPNs were recovered from the soil samples by using the last instar larvae of *C. cephalonica* as insect bait instead of *Galleria mellonella* as used by Bedding and Akhurst (1975). The collected soil samples were processed using the insect baiting technique. Approximately twenty final instar larvae of *C. cephalonica* were placed at the bottom of a glass tube of 100 g capacity (25 x 34 cm) as bait and filled with moistened soil samples. The mouth of the tube was sealed with a Kada cloth and set aside at room temperature (25–28 °C). The tubes were emptied seven days later. The dead larvae were collected using the White's trap method (White, 1927), after 3 days to dissected the insect larvae to assess the multiplication of infective juveniles in larvae. The statistically analysed observations were evaluated for significance in relation to the experiments. Probit analysis (Finney, 1971) was performed on the virulence test data to determine the median lethal concentration (LC_{50}) and median fatal time (LT_{50}). To compare the effectiveness of treatments, the means of all experiments were used. Data on insect mortality percentages were analysed using a multifactor ANOVA, and the Duncan multiple range test was used to separate the means (P>0.05).

RESULTS AND DISCUSSION

Steinernema glaseri was found to be highly virulent against larvae of *A. communis* 2nd instar larvae with lowest LC_{50} values of 30.37 IJ/ larva and minimum time was taken by *A. communis* (35.83 hr/ larva). *Oscheius* sp. was found to be low virulence against *A. communis* with maximum LC_{50} values 41.62 IJ/ larva and LT_{50} 50.18 hr/ larva respectively *Anamola communis* 3rd instar larvae with lowest LC_{50} values of 40.39 IJ/ larva of *S. glaseri* and minimum time was taken by *A. communis* (41.57 hr/ larva) (± 2). *Oscheius* sp. was found to be low virulence against *A. communis* with maximum LC_{50} values 49.75 IJ/ larva and LT_{50} 51.46 hr/ larva respectively The LC_{50} value of the above insect pest was not significantly different from each other as the fiducial limits were overlapping (Table 1). The dosage of nematodes tested 2.5 x 10^9 IJ/ha are found to be effective against *A. communis* on potato under pot culture and field conditions. The insect mortality increased with dosage level and exposure time. The highest larval mortality of 76.66% was observed after 96 hr with *S. glaseri* @ 2.5 x 10^9 IJ/ ha followed by 73.3% reduction larval mortality of *Heterorhabditis* sp 2.5 x 10^9 IJ/ ha. The least mortality (36.6 %) was observed with *Oscheius* sp 2.5 x 10^9 IJ/ ha after 96 hr of exposure time. Similar observation was found to be at 48 and 72 hr of exposure time, the maximum mortality of 23.33 and 56.66% mortality were caused by *S. glaseri* @ 2.5 x 10^9 IJ/ ha. For *Heterorhabditis* sp. 2.5 x 10^9 IJ/ ha which recorded 6.60 and 26.6% reduction respectively. Number of damaged potato tubers by insects was found to decrease with increased dosage of nematodes. *S. glaseri* was found effective than *Heterorhabditis* sp. The minimum tuber damage of 36.66% was detected with *Oscheius* sp. @ 2.5 x 10^9 IJ/ ha.

In the present study, *S. glaseri* and *Heterorhabditis* sp. caused significant reduction to control of white grub population at 60 days after application. However, *S. glaseri* reduced the grub population more effectively compared to *Heterorhabditis* sp. The results of the

Table 1. Pathogenicity of native isolates of EPN against larvae of *A. communis*

Nematode species	Median lethal concentration (LC_{50})						Median lethal time (LT_{50})					
					Fiducial limits						Fiducial limits	
	Chi^2	b	±SE	LC_{50} (IJ/ larva)	Lower	Upper	Chi^2	b	±SE	LC_{50} (IJ/ larva)	Lower	Upper
2nd instar												
Heterorhabditis bacteriophora	0.16	3.73	0.99	38.37	30.47	48.32	0.49	2.69	0.66	40.62	29.31	56.28
H. indica	0.37	3.07	0.97	37.80	28.82	49.57	0.53	319	0.29	45.69	35.08	59.50
Heterorhabditis sp.	0.32	2.59	0.97	33.17	21.40	44.23	0.30	2.94	0.23	41.62	30.89	56.10
Steinernema siamkayai	0.35	2.33	0.97	39.47	28.37	54.91	1.20	2.94	0.22	41.97	31.23	56.40
S. glaseri	0.62	3.25	0.96	30.76	25.04	43.95	0.74	2.89	0.50	35.83	25.74	49.56
S. carpocapsae	0.89	2.63	0.94	32.38	23.02	45.54	1.71	3.23	0.78	61.22	48.82	76.78
Oscheius sp.	1.09	3.02	0.94	41.62	32.06	54.03	1.27	3.55	1.05	50.18	39.90	63.11
3rd instar												
Heterorhabditis bacteriophora	2.14	2.86	0.88	45.60	35.08	59.27	0.42	2.90	0.01	53.67	41.33	69.69
H. indica	1.01	3.14	0.95	41.82	32.43	53.93	0.51	2.99	0.18	46.62	35.56	61.13
Heterorhabditis sp.	3.23	3.26	0.88	46.20	36.59	58.33	0.97	2.74	0.44	45.46	33.82	61.12
Steinernema siamkayai	1.96	3.29	0.92	42.17	33.13	53.69	0.73	2.67	0.60	43.66	31.89	59.77
S. glaseri	0.54	307	0.96	40.39	31.11	52.43	0.51	2.54	0.88	41.57	29.64	58.30
S. carpocapsae	4.56	3.60	0.85	52.68	42.80	64.84	1.76	2.52	0.75	48.01	35.25	65.40
Oscheius sp.	1.23	3.42	0.95	49.75	40.06	61.78	0.38	2.88	0.06	51.46	39.47	67.10

field trial showed that all the dose of nematodes tested 2.5 x 10^9 IJ/ ha were found to be effective against *A. communis* on potato. Observations were made on the % mortality of white grub population, % tuber damage and percentage of healthy tubers and yield. The data revealed that all the treatments had significant effects to control the white grub. *Steinernema glaseri* recorded the highest grub mortality. *Anomala communis* grub mortality was highest (88.33%) after 7 days for *S. glaseri* @ 2.5 x 10^9 IJ/ ha. *S. glaseri* and *Heterorhabditis* sp. were on par with each other with larval mortality of 78.66% for both the nematodes. The similar observation was detected at 4 days interval, with highest larval mortality of 53.33% was caused by *S. glaseri* @ 2.5 x 10^9 IJ/ ha and lowest larval mortality due to *Oscheius* sp. @ 2.5 x 10^9 which recorded at 12.49%. The % tuber damage observed with *S. glaseri* @ 2.5 x 10^9 IJ/ ha was 14.44 and 13.37% respectively compared to control (68.33 %) (Table 2; Fig. 1). The mortality of grub population was 71.33% due to *S. glaseri* and more than 40% due to *Heterorhabditis* sp. The mean % healthy tuber was documented in *S. glaseri* viz., 88.33% and *Heterorhabditis* sp. 80.33% @ 2.5 x 10^9 IJ/ ha respectively. The highest increase in grub mortality and increase in yield over control due to grub mortality was recorded as 14.14 t/ ha and 5.50 t/ ha, respectively in control when *S. glaseri* was applied @ 2.5 x 10^9 IJ/ ha. Treatment with *S. glaseri* at higher dosage of 2.5 x 10^9 IJ/ ha was highly significant over all the treatments, as in this treatment no grub and tuber damage were observed after application (Fig. 1). The nematode species, *S. glaseri*, *S. siamkayai*, *S. carpocapsae*, *H. indica*, *H. bacteriophora*, *Heterorhabditis* sp. and *Oscheius* sp. were successfully multiplied in white grub larvae for the current study, their persistence in the soil was observed even 90 days after application. Additionally, the soil approximate 20% moisture and the temperature 23°C were favourable for the IJs survival and persistence. Mortality of *G. mellonella* larvae in nematode-treated soil samples were assessed for EPN survival.

The present investigation indicated that *S. glaseri* were more virulent to *A. communis*. Virulence of entomopathogenic nematodes was also affected by different larval stages of white grubs as reported by Ma et al. (2013). The maximum larval morality was detected after 96 hr with *S. glaseri* @ 2.5 x 10^9 IJ/ ha. The least mortality was observed with *Oscheius* sp 2.5 x 10^9 IJ/ ha after 96 hr of exposure time. Similar observation was made in white grubs, which showed a did not clear trend for which larval stage was the optimal one for entomopathogenic nematodes and it varied with different entomopathogenic nematodes species and different white grub species (Grewal et al., 2004). Combination of *S. carpocapsae* and *H. indica* had an additive effect over their individual population. *S. carpocapsae* described to perform well against white grub species (Forschler and Gardner, 1991). Sharma and Chandle (2009) reported *S. carpocapsae* is better than *H. indica* for controlling white grubs. This may be due to the better survival and adaptability of *S. carpocapsae* in the soil of the hilly area. Guo et al. (2016) reported that *S. longicaudum* X7 and *H. bacteriophora* HO6 showed good control efficacy against *Holotrichia oblita* larvae, but *H. bacteriophora* HO6 was recommended as a promising agent for white grub control in practice. *S. glaseri* was highly effective against this sedentary pest (Alm et al., 1992). In the environmental conditions are favourable

Table 2. Biocontrol efficacy of native isolates entomopathogenic nematodes against *A. communis* on potato under green house conditions

Treatments	% insect mortality (hr after treatment)			% Tuber damage
	48	72	96	
T_1 - *H. bacteriophora* @ 2.5x10^9 IJs/ ha	11.6± 6.16^{bc}	43.3± 10.46^{ab}	47.3± 11.99^{ae}	59.33± 50.38^{e}
T_2 - *H. indica* @ 2.5x10^9 IJs/ ha	13.3± 6.53^{ab}	33.3± 10.49^{ab}	66.6± 14.95^{ab}	42.66± 40.77^{d}
T_3 – *Heterorhabditis* sp. @ 2.5x10^9 IJs/ ha	23.3± 8.74^{a}	56.6±12.4^{a}	73.3± 16.06^{a}	21.33± 27.49^{b}
T_4 - *S. glaseri* @ 2.5x10^9 IJs/ ha	6.6± 3.91^{c}	26.6± 9.35^{bc}	76.6± 13.33^{c}	18.66± 24.05^{a}
T_5 - *S. siamkayai* @ 2.5x10^9 IJs/ ha	16.6± 7.33^{ab}	33.3± 10.49^{ab}	63.3± 14.56^{b}	31.66± 34.24^{c}
T_6 - *S. carpocapsae* @ 2.5x10^9 IJs/ ha	13.3± 6.53^{ab}	26.6± 9.07^{bc}	46.6± 12.45^{cd}	55.33± 48.06^{e}
T_7 – *Oscheius* sp. @ 2.5x10^9 IJs/ ha	10.0± 5.73^{bc}	16.6± 7.33^{c}	36.6± 11.01^{e}	57.66± 49.41^{e}
T_8 – Control	0± 0.28	0± 0.28	0± 0.28	85.33± 67.60
CD (p=0.05)	2.54	2.50	1.21	3.09
SEd	1.20	1.18	0.57	1.46

Figures in parentheses arc sine transformed values; Column figures followed by different letters significantly different from each other

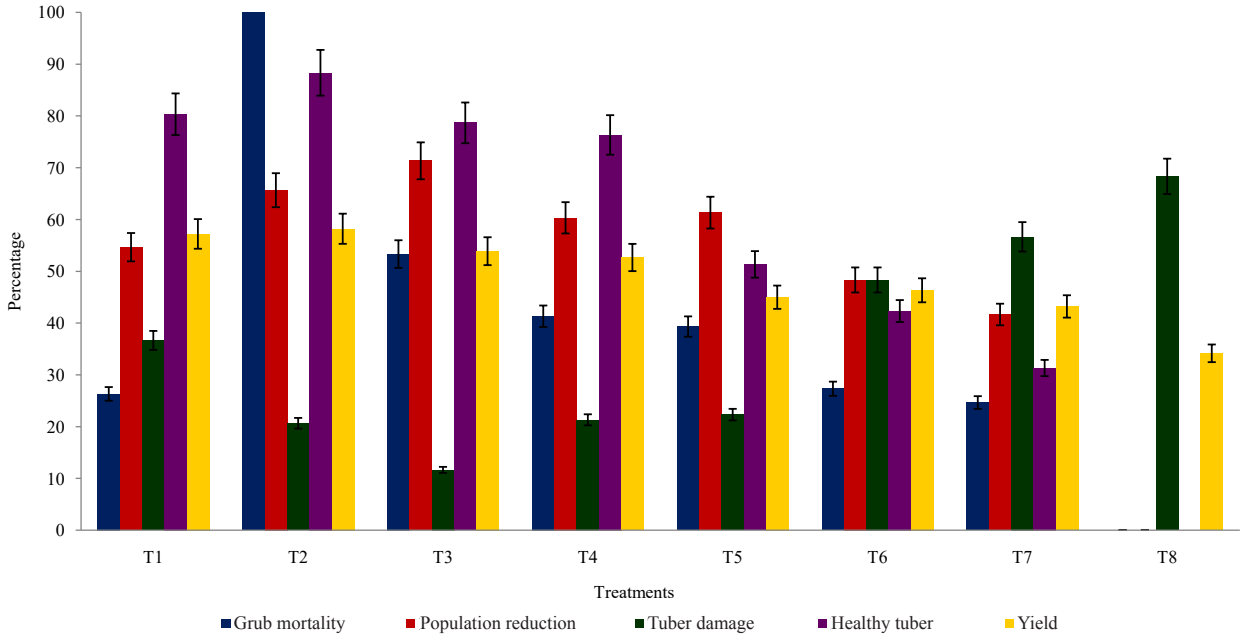


Fig. 1. Virulence of native isolates of entomopathogenic nematodes against *A. communis* on potato under field conditions

for (temperature, moisture, relative humidity and soil type) entomopathogenic nematodes and produce long term effects on pest population (Susurluk and Kumral, 2011).

Field experiment conducted for management at Ooty revealed that population of white grub were reduced by 71.33% when treated with *S. glaseri* followed by *Heterorhabditis* sp. showing 65.66% reduction. Banu et al. (2003) reported mortality of insects with the increased level of entomopathogenic nematodes. The zero mortality of nematode was observed up to 5 days after treatment. Similar result of 35 and 21% mortality was recorded against second instar white grub for *H. indica* and *H. bacteriophora* respectively (Anonymous, 2000). Highest grub mortality was 71.33% after 7 days for *S. glaseri* @ 2.5 x 10^9 IJ/ ha. *S. glaseri* and *Heterorhabditis* sp. @ 2.5 x 10^9 IJ/ ha were on par with each other with larval mortality of 65.66%, respectively. The result of Anupam Sharma et al. (2009) is similar to the present findings which reveals that in field conditions all the dosages of *S. carpocapsae* and *H. indica* (1, 3 and 6x10^5 IJ/ m^2) were effective in reducing the grub population, plant damage as well as tuber damage. Reduction in grub population was 60-80% due to *H. indica* and more than 83% due to *S. carpocapsae*. These observations are related to Koppenhofer and Fusy (2008) reported that controlling white grub with *H. bacteriophora* is safe and highly Integrated Pest Management strategies- compatible with alternative for white grub control. The highest larval mortality of 53.33% was caused by *S. glaseri* @ 5 x 10^9 IJ/ ha and lowest larval mortality was caused due to *Oscheius* sp. @ 12.66t/ ha. Previous work of Georgis and Gaugler (1991) and Hussaini et al. (2005) reported the controlling behaviour of entomopathogenic nematodes expecially *S. carpocapsae* in fields. However the present findings showed that in laboratory, early grub mortality was caused by *S. glaseri* effectively than *H. indica*. Again in the field, *S. glaseri* reduced grubs population more effectively than *H. indica*. White grub management has been strongly dependent on chemical insecticides (Govender, 2007), but entomopathogenic nematodes (EPNs) provide a possible alternative (Grewal et al., 2005).

The entompathogenic nematodes dispersal and persistence in soil, in turn depend upon many abiotic environmental factors, such as soil moisture, temperature and soil texture. More studies have revealed that influence of temperature on the infectivity of entomopathogenic nematodes (El-Sadawy, 2001). The present study suggests that *S. glaseri* is better than *Heterorhabditis* sp. for controlling white grubs. This may be the reason due to better survival at low temperature and adaptability of *S. glaseri* in the soil of the hilly area of Ooty. Entomopathogenic nematode one of the best promising biological control agents for the management of white grubs population (Gitanjali Devi, 2019). Therefore, it is recommended for the bio-intensive management of white grub in potato crop. It is concluded that, biological control can be used as

an alternative to chemical pesticides for the control of various insect pests. The highest larval mortality of 53.33% and 11.66% tuber damage was observed with *S. glaseri* @ 2.5 x 10^9 IJ/ ha under pot culture and field conditions. Infield conditions, *S. glaseri* effectively controlled *Anomala communis* in potato filed. Evaluations of the different nematode species against the early instars (2nd and 3rd) of target white grubs are required, because susceptibility of the different life stage of the grubs varies significantly within the same nematode species, as do different white grub species themselves (Koppenhöfer and Fuzy, 2004). Survival of IJs was assessed directly by counting living IJs and indirectly by baiting with *Galleria mellonella* larvae. The treatments proved to work better at the lowest temperature; however the nematode *S. glaseri* has its best efficacy at the lowest temperature in the field experiment. However, further studies are required to conclude the formulation that can succeed the best results for management of insect pests. In conclusion, baited soil samples showed that global EPN can survive in the soil following treatment. This shows that the applied EPNs were of good quality because they require more energy to last a while in the soil before locating and establishing themselves in the host insects. Additionally, it showed that the way for applying EPNs is into the moist, freshly opened soil furrow application. Applications into the soil are thought to be a beneficial method for using EPNs because they like continuously moist conditions.

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AUTHOR CONTRIBUTION STATEMENT

The authors conducted all experiment and manuscript writing, and A S and B A suggested and supervise the methodology for conducted all experiment and edited the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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ENHANCEMENT OF MATING PERFORMANCE OF STERILE MALES OF MELON FLY *ZEUGODACUS CUCURBITAE* (COQUILLETT) THROUGH METHOPRENE AND CUE LURE

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ABSTRACT

The male pupae of Melon fly, *Zeugodacus cucurbitae* were irradiated at 30 Gy of gamma radiation using Cobalt-60 source. Freshly emerged sterile males (30 Gy) were exposed to different regimes of methoprene and yeast hydrolysate as protein source. Sterile males of 10, 12 and 14 days old were exposed to different regimes of cue lure and yeast hydrolysate. Mating parameters such as lek initiation, lek participation, male calling, mating success and female acceptance index (FAI) were recorded. Sterile males of exposed to methoprene and fed with yeast hydrolysate (M+P+) become sexually matured at three days earlier when compare to protein deprived males (M+P-). High percentage of M+P+ males were engaged in lekking and calling behavior. Sterile males treated with methoprene and fed on protein diet had high percentage of matings with female acceptance index (FAI) of 0.8. Sterile males fed on yeast hydrolysate up to 12-days of age and then accessed to cue lure (P+CL+) had greater mating propensity with frequent mating (43.0%) and FAI of 0.84.

Key words: *Zeugodacus cucurbitae*, methoprene, cue lure, yeast hydrolysate, mating parameters, lek intiation, lek participation, male calling, female acceptance index

Melon fly, *Zeugodacus cucurbitae* (Coquillett) (Tephritidae:Diptera) is an economically most important insect pest of horticultural crops inflicting heavy losses to the horticultural industry in India (Kapoor, 2005). It attacks 61 plant species belonging to 19 different families (De Meyer et al., 2005). Cucurbits are subjected to damage by melon fly right from the primordial stages of the crop up to harvest (Viraktamath et al., 2003). In addition to the direct losses, its economic impacts results in the loss of export markets as well as costly requirements of quarantine restrictions and eradication measures (Badii et al., 2015).

The sterile insect technique (SIT) is a species-specific and environmentally non-polluting method of insect control that relies on the radiation-sterilization of mass reared insects and systematic release of potentiated sterile males into a target area to induce sterility (Knipling, 1979). Factors that can undermine the effectiveness of an SIT programme are poor mating performance of mass reared males as compared to wild males. Improvements in insect quality influence the efficacy of sterile insect technique, because released sterile males have to successfully transfer their sperm carrying dominant lethal mutations to females in the target populations (Hendrichs, 2002). For the success of any SIT programme, males must be able to locate a lekking site, perform a courtship, attract wild females and copulate (Jang et al., 1998). Juvenile hormone (JH) is a pivotal hormone coordinating the development of sexual signaling by enhancing pheromone production and accelerating reproductive maturity in tephritid males (Teal et al., 2000). Methoprene is a juvenile hormone analogue widely used as insect growth regulator and their impact on sexual maturation has been explored in number of tephritid species; such as, Mexican fruit fly *Anastrepha ludens* (Loew), West Indian fruit fly *A. oblique* (Macquart) and Queensland fruit fly *Bactrocera tryoni* (Froggatt) giving young males a mating advantage over non-treated males of the same age (Collins et al., 2015). Cue lure is a parapheromone and its feeding increase mating success in fruit flies (Shelly and Villalobos, 1995). Yeast hydrolysate is the main source of amino nitrogen, minerals and vitamins contributes to male gonadal and accessory glands development and known to influence sexual success in Oriental fruit fly *B. dorsalis* (Hendel), *B. tryoni*, Mediterranean fruit fly *Ceratits capitata* (Wiedemann) (Yuval et al., 2002). Release of enhanced sterile males in target area offers a considerable potential and has been used with great success as a part of an area-wide integrated pest management approach (AW-IPM) against major pests of agricultural and horticultural importance (Vreysen et al., 2006). Keeping these observations in view, the present

investigation was conducted to study the influence of methoprene and cue lure along with yeast hydrolysate on mating behavior of sterile males of *Z. cucurbitae* to identify potential pre-release adult holding diet for enhanced mating performance.

MATERIALS AND METHODS

The experiment was conducted at Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI). New Delhi during 2018-19. The initial culture of melon fly was obtained from infested cucurbit fruits such as bitter gourd, ridge gourd and bottle gourd were collected from the fields of Division of Vegetables Science, ICAR-IARI, New Delhi. Infested fruits were brought to the laboratory and were kept at controlled conditions (25-27°C, 65-75% RH) in 8"×6" glass jars provided with 5 cm thickness of sterilized sand for pupation. The fully-grown larvae pop out from the fruit into soil for pupation. Pupa were collected and transferred to adult rearing cages (30×30×30cm) provided with a mixture of sugar and yeast hydrolysate (3:1) as adult food and water-soaked cotton swabs in 100 ml conical flask as source of water. Cages were cleaned and serviced with adult food and water twice a week. After pre-oviposition period of 10-12 days, equal number of males and females of same age were confined to mating cages. Petri dishes containing semi solid pumpkin fruit substrate covered by a thin parafilm were provided as ovipositional devices to the females to collect the eggs required for the experiment.

The insects required for sterilization were raised by rearing maggots on artificial liquid larval diet 17 (Panduranga et al., 2018). The male pupae of 48 h before adult eclosion were exposed to gamma radiation (cobalt-60 source) dose of 30 Gy at discharge rate of 1.147 kGy/hr using gamma irradiator (GC-5000, BRIT, Mumbai) installed at the Nuclear Research Laboratory, ICAR-IARI, New Delhi. Sterile males were treated topically on the day of emergence with methoprene. Male flies were immobilized by chilling at 5ºC for 5-10 min. One microleter of acetone containing 5 µg of methoprene was applied on dorsal surface of thorax of each male with the help of micropipette. For control males, only 1 µl of acetone was applied. These males were accessed to the diet with or without yeast hydrolysate and treatments as follows: M+ P+: Topical application of methoprene (M), sugar and yeast hydrolysate (P) as adult food; M+ P-: Topical application of Methoprene and only sugar as adult food; M- P+: No methoprene but yeast hydrolysate and sugar as adult food; and M- P-: No methoprene and only sugar as adult food.

Sterile males of 10, 12 and 14 days old fed on adult diet with and without protein (yeast hydrolysate) were exposed to cue lure applied on cotton wick for 2-h before release for mating. The four treatments studied were: P+ CL+: Sugar and protein hydrolysate (P) as adult food followed by cue lure (CL) feeding; P- CL+: Only sugars as adult food followed by cue lure feeding; P+ CL-: Sugar and protein hydrolysate as adult food and no cue lure feeding; and P- CL-: Only sugar as adult food and no cue lure feeding. Flies from each treatment were maintained in a separate adult rearing cage at 25-27 ºC and 65-70% RH with photoperiod of 12:12 h (L:D). Virgin untreated females used in the experiments were maintained in the absence of males in separate cage provided with mixture of sugar and yeast hydrolysate (3:1) and water at ad libitum.

Lek initiation, lek participation, and male calling behaviors of sterile males in all the treatments were recorded up to 12 days of age. Observations on these parameters were recorded at every 10 min interval during 5.00-8.00 pm under semi-dark conditions. Lek initiation is the first male started to emit pheromone (wing vibration and anus beating) in a certain area of cage, resulting in initiation of aggregations (Kuba and Sokei, 1988). Lek participation is the number of males that joined the first male for calling (Iwahashi and Majima, 1986). Male calling is the number of males calling in a lek (expansion of pleural regions of the abdomen and eversion of glistening rectal glands to emit pheromones). Mating success is the number of males that achieved copulation. Female acceptance index (FAI) is number of successful matings divided by total number of males attempted to mate. For recording mating success and FAI, untreated virgin females of same age (12-days old) were released to each treatment. The number of matings was recorded at every 10 min interval and mating pairs were collected in a glass vial. FAI was calculated by dividing the number of successful matings by total number of males attempted to mate. These observations were recorded up to 20 days of age. In case of cue lure experiment, mating parameters were recorded for 5-days following cue lure exposure. Data obtained were calculated as mean± standard error (SE). Mean values were obtained from three replications. Data were subjected to analysis ANOVA with the honestly significant difference (HSD) value calculated as Tukey's statistic at α=0.05 using SAS version 9.4 available at ICAR-IASRI, New Delhi, India.

RESULTS AND DISCUSSION

Mating parameters such as mating success female acceptance lek initiation, lek participation and male calling behavior of sterile males of melon fly exposed to different regimes of methoprene and yeast are presented in Table 1. Sterile males that were exposed to methoprene and allowed to fed on protein diet (M+P+) formed leks early and participated in male calling in comparatively shorter time. At 5 DAT, 12% of M+P+ males initiated leks, 16% joined the leks and nearly 11% of males were engaged in male calling and on next day (i.e. 6 DAT), 26.7, 25.3 and 21.3% of males were involved in lek initiation, lek participation and male calling, respectively. Whereas the male flies from the remaining treatments have initiated leks on 8th day onwards except M-P+. The sterile males from M-P+ reached sexual maturity at 7 DAT as 9.3, 12.0 and 8.0% of males were involved in lek initiation, lek participation and male calling behavior, respectively. However, significantly high percentage of M+P+ males initiated leks (40.0) and participated in leks (32.0) and nearly 31.0% of flies were engaged in male calling at 7 DAT. Involvement and participation of M+P+ males in lek intiaiton, lek participation and male calling behaviours increased with increase in age of the flies. It was also observed that the sterile males from M+P- and M-P- exhibited mating behaviour from the age of 8-days onwards and slightly increased their performance up to the age of 10-days. While on 11 and 12-days of age, least number of males was involved in mating behavior due to lack of protein in their diet. More than 50% of M+P+ males initiated and participated in leks and more than 45% of males were involved in male calling at 8 DAT. Male files that were not treated with methoprene (M-P+) showed quite low activity on 8th day as 22.7%, 18.7%, and 12.0% of males were involved in lek initiation, lek participation and male calling behavior, respectively. The percentage of M-P+ sterile males involved in lek initiation, lek participation and male calling was increased (ranged between 30.07-48.7, 28.0-30.7, 21.3-36.0%, respectively) at 11-days of age. Whereas in M-P- and M+P-; the percentage of males involved in lek initiation (16.0 and 10.7%), lek participation (14.7 and 12.0%) and male calling behaviors (10.7 and 9.3%) were not significantly differ, respectively (Table 1).

The sterile males subjected to methoprene application and then access to adult diet consist of yeast hydrolysate and sugar (1:3 parts) showed increasing trend in performance of mating behaviour up to 11-days of age. The percentage of male involvement in lek initiation, participation and male calling (at 9-11 DAT) was ranged between 62.7-72.0%, 54.7-60.0% and 50.7-61.3%, respectively. The next best treatment was M-P+, in which the sterile males involved in lek initiation, lek participation and male calling was increased (ranged between 30.07-48.7, 28.0-30.7, 21.3-36.0%, respectively) at 11-days of age. Whereas at 12-days of age, mating performance of M+P+ and M-P+ males was reduced. The males that were exposed to methoprene and then access to the diet containing yeast hydrolysate and sugar (M+P+) achieved maximum matings throughout the experiment, compared to the remaining treatments. The mating success by M+P+ males was in the increasing trend up to 18 DAT ranging from 26.7 (13 DAT) to 44.0% (18 DAT), Whereas at 19 and 20 DAT; the mating success decreased to 34.7 and 30.7%, respectively. It was followed by M-P+ with mating success ranging from 17.3 (13 DAT) to 26.7% (18 DAT). Whereas in the treatments; M+P- and M-P, matings were significantly lower than M+P+ males. However, the mating success increased from 6.7 to 14.7% and 10.7 to 21.3%, respectively from 13 to 16 DAT and were on par with each other. In the treatments; M+P- and M-P-, the mating gradually decreased at 17 DAT onwards. Where as in M-P+, mating success started declining at 19 DAT. The maximum mating (41.3 and 44%) was achieved by M+P+ males at 17 and 18 DAT, respectively with FAI of 0.88. But at 19 and 20 DAT, the mating success of M+P+ males with untreated females showed decreasing trend i.e. 34.7 (FAI:0.64) and 30.7% (FAI:0.51), respectively (Table 1).

The males treated with methoprene and fed with protein diet (M+P+) had FAI of >0.8, which indicates that males that were engaged in calling behavior are highly potent and readily accepted by the females for mating. The FAI for M-P+ males was ranged from 0.59-0.77 at 13 to 16 DAT, respectively. Where as in M-P- and M+P-, the males harboured FAI of 0.48-0.65 and 0.39-0.43, respectively and were at par with each other (Table 1).

Effect of protein feeding and cue lure exposure on mating behavior of 10, 12 and 14 days old sterile males of *B. cucurbitae* are presented in Table 2. Significantly misl sterile males from P+CL+ were involved in lek initiation (54.7), lek participation (43.3) and male calling (42.7) on 1-day after exposure to cue lure compared to the other treatments. In P+CL-; 32.0% of sterile males initiated leks and 26.7% of sterile males had participated in leks, where as 24.0% of males called for mates. In P-CL+, very low percentage of

Table 1. Influence of methoprene and adult dietary protein on *B. cucurbitae* sterile males

Treatment	Lek initiation (%)	Lek participation (%)	Male calling (%)	Mating success (%)	Female acceptance index (FAI)
	5 DAT			13 DAT	
M+P+	12.0a (6.89± 1.33)	16.0a (9.21± 1.34)	10.7a (6.12± 0.76)	26.7a (15.46± 0.79)	0.87a (0.49± 0.04)
M+P-	0.0b	0.0b	0.0b	6.7b (3.82± 0.76)	0.39b (0.22± 0.03)
M-P+	0.0b	0.0b	0.0b	17.3ab (9.98± 1.54)	0.59ab (0.33± 0.04)
M-P-	0.0b	0.0b	0.0b	10.7b (6.12± 2.03)	0.48b (0.27± 0.04)
	6 DAT			14 DAT	
M+P+	26.7a (15.47± 1.59)	25.3a (14.67± 0.79)	21.3a (12.32± 0.78)	30.07a (17.87± 2.11)	0.87a (0.49± 0.03)
M+P-	0.0b	0.0b	0.0b	8.0b (4.58± 1.32)	0.47b (0.26± 0.08)
M-P+	0.0b	0.0b	0.0b	18.7ab (10.78± 2.81)	0.68ab (0.38± 0.02)
M-P-	0.0b	0.0b	0.0b	13.3b (7.66± 1.53)	0.50ab (0.28± 0.02)
	7 DAT			15 DAT	
M+P+	40.0a (23.58± 1.44)	32.0a (18.67± 1.39)	30.7a (17.88± 2.11)	33.3a (19.53± 3.27)	0.86a (0.49± 0.04)
M+P-	0.0c	0.0c	0.0c	10.7b (6.12± 2.03)	0.42b (0.24± 0.06)
M-P+	9.3b (5.35± 0.76)	12.0b (6.89± 1.33)	8.0b (4.59±1.32)	20.0ab (11.53± 1.35)	0.68ab (0.38± 0.03)
M-P-	0.0c	0.0c	0.0c	13.3b (7.66± 0.77)	0.57ab (0.32± 0.01)
	8 DAT			16 DAT	
M+P+	59.3a (36.42± 1.72)	53.3a (32.40± 3.98)	45.3a (27.08± 3.77)	38.7a (22.77± 2.18)	0.83a (0.47± 0.02)
M+P-	10.7c (6.13± 2.03)	12.0b (6.89± 1.33)	9.3b (5.36± 1.53)	14.7b (8.44± 2.04)	0.43b (0.24± 0.03)
M-P+	22.7b (13.11± 2.07)	18.7b (10.77± 2.05)	12.0b (6.89± 1.33)	25.3ab (14.72± 3.45)	0.77a (0.44± 0.03)
M-P-	16.0bc (9.21± 1.34)	14.7b (8.44± 1.55)	10.7b (6.12± 0.77)	21.3ab (12.35± 3.15)	0.65ab (0.37± 0.04)
	9 DAT			17 DAT	
M+P+	62.7a (38.85± 1.99)	54.7a (33.19± 2.39)	50.7a (30.49± 2.33)	41.3a (24.42± 1.66)	0.88a (0.50± 0.01)
M+P-	12.0c (6.89± 1.33)	14.7c (8.44± 2.04)	13.3b (7.66± 0.77)	12.0c (6.89± 1.33)	0.36c (0.20± 0.02)
M-P+	30.7b (17.87± 1.61)	28.0b (16.26± 1.37)	21.3b (12.32± 0.78)	26.7b (15.48± 2.09)	0.72ab (0.41± 0.01)
M-P-	24.0b (13.88± 1.36)	20.0bc (11.54± 1.35)	14.7b (8.44± 2.04)	20.0bc (11.53± 2.31)	0.54bc (0.30± 0.03)
	10 DAT			18DAT	
M+P+	67.3a (42.46± 3.13)	57.3a (35.18± 4.12)	53.3a (32.33± 3.22)	44.0a (26.21± 3.85)	0.80a (0.45± 0.01)
M+P-	18.7c (10.76± 0.77)	20.0b (11.54± 1.35)	16.0b (9.21± 1.34)	12.0b (6.90± 4.62)	0.28c (0.16± 0.03)
M-P+	34.7b (20.30± 2.14)	26.7b (15.47± 1.59)	28.0b (16.26± 1.37)	26.7ab (15.46± 1.33)	0.47b (0.26± 0.02)
M-P-	29.3bc (17.05± 0.80)	21.3b (12.34± 2.81)	24.0b (13.91± 2.73)	17.3b (9.99± 3.53)	0.34bc (0.19± 0.01)
	11 DAT			19DAT	
M+P+	72.0a (46.10± 1.91)	60.0a (36.96± 2.81)	61.3a (37.97± 3.44)	34.7a (20.35± 3.54)	0.64a (0.36± 0.01)

(*contd...*)

(*contd...*)

M+P-	13.3d (7.66± 0.77)	14.7c (8.44± 2.04)	10.7c (6.12± 0.76)	9.3b (6.90± 1.33)	0.13c (0.07± 0.02)
M-P+	48.7b (29.12± 1.16)	30.7b (17.88± 2.11)	36.0b (21.10± 1.41)	22.7b (13.10± 2.67)	0.31b (0.17± 0.02)
M-P-	26.7c (15.48± 2.09)	18.7bc (10.78± 2.82)	22.7c (13.11± 2.07)	13.3b (7.66± 2.67)	0.22bc (0.12± 0.00)
	12 DAT			20DAT	
M+P+	66.7a (41.91± 2.69)	52.0a (31.35± 1.99)	56.0a (34.07± 2.31)	30.7a (17.87± 2.11)	0.51a (0.29± 0.01)
M+P-	10.7c (6.12± 0.76)	12.0c (6.89± 1.33)	9.3c (5.35± 0.76)	9.3b (5.35± 0.76)	0.09b (0.05± 0.00)
M-P+	30.7b (17.90± 2.91)	22.0b (12.72± 1.78)	20.0b (11.56± 2.70)	17.3b (9.98± 0.77)	0.19b (0.10± 0.00)
M-P-	16.0c (9.21± 1.34)	13.3c (7.66± 0.77)	10.7bc (6.12± 0.77)	12.7b (7.66± 1.53)	0.13b (0.07± 0.01)

Within a column, means followed by the same letter are not significantly different (α=0.05; Tukey test, PROC ANOVA).
Figures in parentheses are arc sine transformed values ± standard error, DAT: Days after treatment.

sterile males i.e. 14.7, 10.7, and 8.0 were involved in lek initiation, lek participation and male calling, respectively. The sterile males from P-CL- performed better in terms of lekking and calling behaviour compare to P-CL+ males.

The successful matings (38.7) was significantly high in P+CL+ with FAI of 0.80. While in P+CL, P-CL- and P-CL+, the mating success were 10.7, 8.0 and 5.3 with FAI of 0.38, 0.33 and 0.31, respectively and these three treatments were on par with each other. At 2-day after exposure to cue lure, sexual performance of P+CL+ sterile males gradually declined and insignificant difference was observed from P+CL- males. Nearly 43.0 and 32.0% of P+CL+ males initiated and participated in leks, where as in P+CL-, 38.7 and 28.0% of sterile males were involved in initiation and participation of leks, respectively. In P+CL+ and P+CL-, 33.3 and 29.3% of males were engaged in emission of pheromones (male calling), respectively. The mating success in all the treatments was increased at 11 days of age, when compared to 10 days of age. The sterile males from P+CL+ achieved high of mating success (30.3). At 3-days after exposure, the mating parameters recorded for all the four treatments continued to decline. Further, the P+CL+ males involved in lek initiation, lek participation, male calling, and mating success reduced from 34.7, 25.3, 26.7 and 21.3% (at 3 DAT) to 17.3, 12.0, 13.3 and 13.3% (at 5 DAT). These results indicated that, P+CL+ sterile males showed better performance and were effectively involved in all the mating parameters at only one day of exposure to cue lure. From the second day onwards, sexual performance of P+CL+ males were similar with the remaining treatments (Table 3).

Sterile males fed with yeast hydrolysate and had accessed to cue lure (P+CL+) at 1 DAT were actively involved in forming leks (60.0%) and nearly 50.0% of males participated in leks and 45.3% called for mates. Significantly lower percentage i.e. 38.7, 29.3 and 32.0 of P+CL- males were involved in lek initiation, participation and male calling, respectively. P+CL+ males had successful matings (43.0%) with FAI of 0.84. While in the remaining treatments, the mating success was in the range of 8.0-17.3 % with FAI of 0.28-0.45 only. The mating potency of P+CL+ sterile males started declining 2-day after exposure to cue lure, as the percentage of male involvement in lek initiation, participation and calling behaviour was reduced to nearly ¾ times and does not remain significantly different from those sterile males which were fed with amino nitrogen molecules but never exposed to synthetic cue lure in the laboratory (P+CL-), however, their mating success was 36.0% with the FAI of 0.65. Less percentage of P-CL+ males were involved in mating, compare to P-CL- males. The sexual performance of sterile males keeps on decreasing with increase in the period of the treatment of flies. The percentage of lek initiation, lek participation, male calling and mating success decreased from 35.7 to 18.7, 24.0-11.7, 24.0-9.3 and 32.0 -21.3% from 3 DAT to 5 DAT, respectively, in P+CL+ sterile males. The similar trend was observed in all the treatments also. These results indicate that, an exposure of sterile males fed with yeast hydrolysate and cue lure had enhanced the mating behaviour for almost one day by increasing their ability in forming leks and emission of pheromones to attract their female mates for successful matings (Table 2).

At one day after exposure, nearly 47.0% of P+CL+ males initiated leks, to which about 36.0% of males joined and 30.7% were involved in calling. In P+CL- treatment, 25.3% of sterile males were involved in lek

Table 2. Influence of adult dietary protein and cue lure on mating behavior of 10-14 days old sterile males of *B. cucurbitae*

Treatment	Lek initiation (%)	Lek participation (%)	Male calling (%)	Mating success (%)	FAI	Lek initiation (%)	Lek participation (%)	Male calling (%)	Mating success (%)	FAI	Lek initiation (%)	Lek participation (%)	Male calling (%)	Mating success (%)	FAI
	10 Days Old					12-days old					14-days old				
						1 DAT									
P+CL+	54.7a (33.19± 2.40)	43.3a (25.68± 1.54)	42.7a (25.27± 1.70)	38.7a (22.75± 4.62)	0.80a (0.46± 0.01)	60.0a (37.0± 3.32)	49.7a (29.64± 3.14)	45.3a (26.99± 2.28)	43.0a (25.62± 2.67)	0.84a (0.48± 0.03)	46.7a (27.85± 2.27)	36.0a (21.10± 1.42)	30.7a (17.87± 2.12)	32.0a (16.26± 1.38)	0.77a (0.44± 0.01)
P-CL+	14.7c (8.43± 1.55)	10.7c (6.12± 2.03)	8.0c (4.58± 1.33)	5.3b (3.05± 0.77)	0.31b (0.17± 0.03)	17.3c (9.98± 0.78)	9.3c (5.35± 0.77)	10.7c (6.12± 0.77)	8.0b (4.60± 1.33)	0.28c (0.15± 0.01)	16.0b (9.20± 1.34)	9.7b (5.35± 0.77)	9.3b (5.35± 0.77)	5.3b (3.05± 0.77)	0.17c (0.09± 0.01)
P+CL-	32.0b (18.66± 1.40)	26.7b (15.47± 1.59)	24.0b (13.88± 1.36)	10.7b (6.12± 0.77)	0.38b (0.21± 0.02)	38.7b (22.75± 1.67)	29.3b (17.06± 1.59)	32.0b (18.66± 1.40)	17.3b (9.98± 0.78)	0.45b (0.25± 0.02)	25.3b (14.68± 1.57)	16.0b (9.20± 1.34)	16.0b (9.20± 1.34)	9.3b (5.35± 0.47)	0.35b (0.20± 0.02)
P-CL-	21.3bc (12.31± 0.78)	13.3c (7.66± 0.77)	12.0c (6.89± 1.33)	8.0b (4.58± 1.33)	0.33b (0.18± 0.02)	28.0bc (16.26± 1.38)	17.3bc (9.98± 1.55)	20.0c (11.53± 1.35)	12.0b (6.89± 1.33)	0.31c (0.17± 0.01)	20.0b (11.53± 1.35)	14.7b (8.43± 1.55)	12.0b (6.89± 1.33)	6.7b (3.82± 0.38)	0.22c (0.12± 0.02)
						2 DAT									
P+CL+	42.7a (25.28± 2.23)	32.0a (18.66± 1.40)	33.3a (19.46± 0.81)	30.3a (17.91± 3.49)	0.70a (0.40± 0.01)	44.0a (26.23± 3.97)	34.7a (20.33± 2.96)	32.0a (18.70± 2.80)	36.0a (21.14± 2.84)	0.65a (0.40± 0.03)	32.0a (18.66± 1.40)	22.7a (13.10± 1.57)	22.7a (13.11± 2.07)	29.3a (17.05± 0.80)	0.61a (0.34± 0.01)
P-CL+	12.0c (6.89± 1.33)	9.3c (5.35± 0.77)	6.7c (3.82± 0.77)	8.7b (4.58± 1.33)	0.32c (0.18± 0.02)	13.3c (7.66± 1.33)	10.7b (6.12± 0.77)	8.0c (4.58± 0.00)	10.7b (6.12± 0.77)	0.32b (0.18± 0.03)	12.7b (7.66± 0.77)	8.0b (4.58± 1.33)	8.0b (4.60± 0.01)	8.0c (4.58± 1.33)	0.24c (0.13± 0.03)
P+CL-	38.7a (22.75± 1.67)	28.0ab (16.26± 1.38)	29.3a (17.05± 0.80)	18.7ab (9.20± 1.34)	0.54ab (0.30± 0.03)	30.7ab (17.86± 1.61)	21.3b (12.31± 0.78)	21.3ab (12.31± 0.78)	22.7ab (13.11± 2.07)	0.51ab (0.29± 0.03)	21.3ab (12.33± 2.07)	13.3b (7.66± 0.77)	13.3ab (7.66± 0.77)	15.7b (10.75± 0.78)	0.49ab (0.28± 0.04)
P-CL-	25.3b (14.67± 0.79)	17.3bc (9.99± 2.06)	17.0b (9.98± 0.78)	12.0b (6.89± 1.33)	0.40bc (0.22± 0.01)	21.3bc (12.31± 0.78)	17.3b (9.98± 0.78)	14.7bc (8.43± 0.77)	14.7b (8.43± 0.77)	0.39b (0.22± 0.01)	16.0b (9.20± 1.34)	9.3b (5.35± 0.77)	12.0b (6.90± 1.33)	13.3bc (7.66± 2.04)	0.33bc (0.18± 0.04)
						3 DAT									
P+CL+	34.7a (20.28± 0.81)	25.3a (14.67± 0.79)	26.7a (15.48± 2.09)	21.3a (12.33± 2.07)	0.52a (0.29± 0.02)	35.7a (20.33± 2.96)	24.0a (13.91± 2.73)	24.0a (13.91± 2.73)	32.0a (18.66± 1.40)	0.49a (0.28± 0.03)	28.0a (16.30± 2.37)	20.0a (11.53± 1.35)	14.7a (8.43± 1.55)	20.0a (11.53± 2.35)	0.40a (0.23± 0.03)
P-CL+	9.3c (5.35± 1.53)	5.3c (3.05± 0.77)	5.3b (3.05± 0.77)	8.0a (4.58± 1.33)	0.27c (0.15± 0.02)	10.7b (6.12± 0.77)	8.0b (4.58± 1.33)	5.3b (3.05± 0.77)	9.3c (5.35± 0.77)	0.22c (0.12± 0.01)	6.7b (3.82± 0.77)	5.3b (3.05± 0.77)	4.0b (2.29± 1.32)	6.7b (3.82± 0.77)	0.16c (0.09± 0.01)

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P+CL-	28.0ab (16.26± 1.38)	17.3ab (9.98± 1.55)	21.3a (12.33± 2.07)	16.7a (10.76± 2.05)	0.46ab (0.26± 0.01)	25.3ab (14.69± 2.10)	18.7ab (10.77± 2.05)	14.7ab (8.43± 1.53)	21.3b (12.31± 0.78)	0.39ab (0.22± 0.01)	14.7ab (8.45± 2.79)	10.7b (6.12± 0.77)	12.0ab (6.90± 1.33)	13.3ab (7.66± 0.77)	0.32ab (0.18± 0.02)
P-CL-	22.7b (13.11± 2.07)	12.0bc (6.89± 2.30)	14.7ab (8.43± 0.77)	12.0a (6.89± 1.33)	0.37bc (0.21± 0.02)	14.7b (8.43± 0.77)	12.0ab (6.89± 1.33)	8.0b (4.58± 1.33)	12.0c (6.90± 1.33)	0.32bc (0.18± 0.02)	12.0b (6.89± 1.33)	6.7b (3.82± 0.77)	9.3ab (5.35± 0.77)	10.7ab (6.12± 0.77)	0.19bc (0.10± 0.01)
							4 DAT								
P+CL+	28.0a (16.26± 1.38)	22.7a (13.09± 0.78)	20.0a (11.53± 1.35)	18.7a (11.53± 1.35)	0.52a (0.29± 0.03)	26.7a (15.47± 1.59)	18.7a (10.75± 0.78)	18.7a (10.75± 0.78)	26.7a (15.47± 1.59)	0.40a (0.23± 0.02)	10.7a (6.12± 0.77)	12.0a (6.89± 1.33)	9.3a (5.35± 0.77)	17.3a (9.99± 2.06)	0.35a (0.20± 0.02)
P-CL+	8.0b (4.58± 1.33)	2.7c (1.52± 0.76)	4.0b (2.29± 0.00)	6.7c (3.82± 0.77)	0.25c (0.14± 0.02)	5.3c (3.05± 0.77)	2.3b (1.52± 0.76)	2.7c (1.52± 0.76)	5.3b (3.05± 0.77)	0.28bc (0.14± 0.02)	4.0a (2.29± 1.32)	2.7b (1.53± 0.76)	1.3b (0.76± 0.01)	4.0b (2.30± 1.32)	0.07c (0.03± 0.02)
P+CL-	20.0ab (11.53± 1.35)	14.7b (8.43± 0.77)	13.3ab (7.66± 1.54)	16.0ab (9.21± 1.34)	0.42ab (0.24± 0.01)	22.7ab (13.11± 2.07)	13.3a (7.66± 0.77)	12.0ab (6.89± 1.33)	20.0a (11.53± 1.35)	0.32ab (0.18± 0.01)	8.0a (4.58± 1.33)	9.3ab (5.35± 0.75)	5.3ab (3.05± 0.77)	12.0ab (6.90± 1.33)	0.25ab (0.14± 0.01)
P-CL-	13.3b (7.66± 2.04)	8.0bc (4.58± 1.33)	9.3b (5.35± 1.53)	9.3bc (5.35± 0.77)	0.30bc (0.17± 0.02)	12.0bc (6.89± 1.33)	6.7b (3.82± 0.77)	6.7bc (3.82± 0.77)	9.7b (5.35± 0.77)	0.21c (0.12± 0.01)	6.7a (3.82± 0.77)	4.0b (2.30± 0.00)	4.0b (2.29± 0.00)	5.3b (3.05± 0.77)	0.16bc (0.09± 0.02)
							5 DAT								
P+CL+	17.3a (10.00± 2.79)	12.0a (6.89± 2.20)	13.3a (7.66± 2.04)	13.3a (10.75± 0.78)	0.38a (0.21± 0.02)	18.7a (10.75± 0.78)	11.7a (6.89± 1.33)	9.3a (5.35± 0.77)	21.3a (12.31± 0.78)	0.30a (0.17± 0.02)	8.0a (4.58± 1.33)	6.7a (3.82± 0.77)	6.4a (3.82± 0.77)	14.7a (8.43± 1.55)	0.26a (0.14± 0.01)
P-CL+	2.7b (1.52± 0.76)	1.3a (0.76± 0.01)	2.7b (1.52± 0.76)	4.0b (2.29± 1.32)	0.11b (0.06± 0.03)	3.7c (1.53± 0.76)	2.7b (1.53± 0.76)	1.3b (0.76± 0.01)	2.7c (1.52± 0.76)	0.05c (0.03± 0.01)	2.7a (1.52± 1.35)	1.3b (0.76± 0.01)	1.3b (0.76± 0.04)	1.3b (0.76± 0.09)	0.02b (0.01± 0.01)
P+CL-	13.3ab (7.66± 1.54)	8.0a (4.58± 1.33)	9.3ab (5.35± 0.77)	10.7ab (6.12± 0.77)	0.33a (0.19± 0.01)	15.3ab (7.70± 0.77)	10.7a (6.12± 0.77)	6.7ab (3.82± 0.77)	13.3b (7.66± 0.77)	0.24ab (0.13± 0.01)	5.3a (3.05± 0.77)	4.0ab (2.29± 0.00)	4.0ab (2.30± 0.00)	6.7ab (3.82± 0.77)	0.14ab (0.08± 0.02)
P-CL-	8.0ab (4.58± 1.33)	2.7a (1.52± 0.76)	2.7b (1.52± 0.76)	6.7b (3.82± 0.77)	0.24ab (0.13± 0.01)	9.3b (5.35± 0.77)	5.3ab (3.05± 0.77)	5.3ab (3.05± 0.77)	5.3c (3.05± 0.77)	0.17bc (0.09± 0.02)	5.3a (3.05± 0.77)	2.7ab (1.52± 0.76)	2.7ab (1.52± 0.76)	4.0b (2.30± 1.32)	0.06b (0.03± 0.01)

Within a column, means followed by the same letter are not significantly different (α=0.05; Tukey test, PROC ANOVA). Figures in parentheses are Arc Sine transformed values ± standard error.

initiation and 16.0% each in lek participation and male calling. The mating behaviour of P-CL- and P-CL+ sterile males was not significantly different from P+CL- males. Significantly high percentage of mating success (32.0%) was recorded from P+CL+ compare to P+CL-(9.3%), P-CL-(6.7%) and P-CL+(5.3%). The FAI (0.77) was high for P+CL+ males followed by P+CL- (0.35). At 2-days after exposure, lekking and calling abilities of sterile males from all the four treatments decreased and continued to decline. In P+CL+, the performance of sterile males in initiation and participation of leks decreased from 32.0 to 8.0% and 22.7-6.7%, respectively during the observation period ranged from 2 DAT to 5 DAT. Similarly the percentage of male calling and mating success were also decreased to 6.4 and 14.7. The percentage of lek initiation, lek participation, male calling and mating success by P+CL- males decreased at 2-DAT i.e. from 21.3, 13.3, 13.3 and 18.7% to 5.3, 4.0, 4.0, and 6.7% at 5-DAT, respectively. The mating parameters recorded for P-CL- and P-CL+ males were statistically on par with P+CL- throughout the experiment. However, the least percentage of P-CL+ males participated in lekking and calling behaviours (Table 2).

The sterile males applied with methoprene on the day of emergence and accessed to adult diet consists of yeast hydrolysate (M+P+) attained sexual maturity at the age of 5-days. Sterile males from M+P+ has initiated and participated in leks by calling their mates at 2-days earlier than the males from M+P-, M-P+ and M-P- treatments. These results are in agreement with the findings of Collins et al. (2015) who reported that exposure of adult flies of *B. tryoni* to methoprene and having access to the diet containing yeast hydrolysate advanced the sexual maturation for 2-days. Sterile males of *A. fraterculus* treated with methoprene attained sexual maturity earlier than untreated males and exhibited lekking behaviour and attractiveness to females as mature wild males (Segura et al., 2009; Liendo et al., 2013). The sterile males from M+P- and M-P- started participation in mating behaviour at 8-DAT and slightly increased their performance up to the age of 10-days. While on 11 and 12-days of age, least number of males was involved in mating behaviour due to lack of protein in their diet. Sterile males of *B. tryoni* fed on protein diet had higher levels of mating propensity than sugar fed males (Perez-Staples et al., 2008).

In the present study, high percentage of M+P+ sterile males had initiated (57.0) and participated (48.0) in leks. Similar results were reported by Yuval et al. (1998) that application of methoprene and exposure to protein increased male lek initiation and participation in *C. capitata*. The males that were accessed to protein without methoprene application (M-P+) performed better than M-P- and M+P- (protein deprived) males. Nearly 36.0 and 26.0% of protein fed sterile males (M-P+) involved in lek initiation and lek participation (Fig. 1). These results are comparable with the earlier reports that sterile males of *C. capitata* fed on protein diet are more likely to start and participate in leks compare to the males fed on protein deprived diet (Taylor and Yuval, 1999; Joachim-Bravo, 2009). The reason could be that protein-rich diet increased the sexual signaling of methoprene treated sterile males, in which 43.0% of M+P+ males were engaged in emission of pheromones to attract their mates. Methoprene treated and protein fed sterile males were more attractive to their mates with female acceptance index (FAI) of 0.82 at which M+P+ males achieved higher mating success(35.0%). Bachmann et al. (2017) also opined that young and aged females tended to mate more frequently with methoprene treated-males as they released larger amounts of pheromonal compounds *A. fraterculus*. Similar results were reported in *C. capitata* that methoprene and protein showed additive effect which enhanced the pheromone calling and mating success (Yuval et al., 1998).

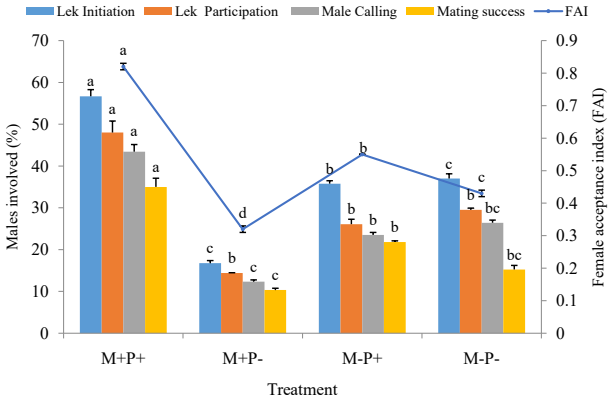


Fig. 1. Sexual performance of sterile males of *B. cucurbitae* exposed to methoprene (M+/M-) and adult dietary protein (P+/P-)

Only protein fed sterile males (M-P+) called earlier and had frequent matings (22.0%) as compared to males fed on sugar alone (i.e. M-P-) or treated with methoprene only (i.e. M+P-). It clearly shows that the diet containing yeast hydrolysate significantly increased the male signaling to attract females for mating. Aluja et al. (2001) found that males of *A. ludens* fed on combination of protein and dry sucrose were sexually active and had higher mating success. Sterile males of

C. capitata fed on diet containing yeast hydrolysate expended more energy during male calling than males fed on sucrose only (Warburg and Yuval, 1997; Kaspi et al., 2000). Role of protein on mating success of sterile males of *C. capitata* and found that protein fed sterile males secured highest copulation rates than the males fed on protein deprived diet (Joachim-Bravo et al., 2009). Sterile males of *B. tryoni* fed on protein diet achieved higher levels of mating probability and longer copulations than males fed on protein-deprived diet (Perez-Staples et al., 2007).

In the present study, application of methoprene and access to the protein (yeast hydrolysate) diet had showed a synergistic effect on mating behaviour of sterile males of melon fly. Hence, M+P+ males had initiated and participated more in aggregations. Synergistic interaction of methoprene and protein could accelerate the sexual maturity in sterile males of *Z .cucurbitae* and enhanced their mating success. Males of *A. suspensa* exposed to methoprene and hydrolyzed yeast (M+P+) emitted pheromones and attracted more females and had more successful mating than males of other treatments (Pereira et al., 2009) 31. Males of *Z. cucurbitae* from M+P+ treatment had higher mating propensity up to the age of 18-days. These results were on par with the report of Shelly et al. (2005) found that males of *B. dorsalis* fed on yeast hydrolysate up to 12-days had mating propensity up to 21 days of age. Recently, Bachmann et al. (2017) found that exposure of *A. fraterculus* males to methoprene and protein during pre-maturation period enhanced the male mating success by increased pheromone production. Further they found that methoprene treatment enhanced male sexual competitiveness even after the sexual maturation phase, and the effect did not decrease until males were older than 20 days.

The existence of male lures was reported approximately 100 years ago and most widely used in detection and management of tephritids. Typically, males emit pheromone to attract females and recent evidence indicates that naturally occurring male lures may function as precursors in pheromone synthesis. The idea that synthetic parapheromones mimic the male sex pheromone appears reasonable and shown to be valid (Wee et al., 2007).

Cue lure (4-[p-Acetoxyphenyl]-2-butanone) is a parapheromone and highly attractive to *B. curcubitae* males. Sterile males of *B. cucurbitae* fed with yeast hydrolysate during pre-copulatory period of 10-days and then exposed to cue lure (P+CL+) had mating advantage over the males fed on either sugar only (P-CL-) or cue lure alone (P-CL+). It was followed by the males (P+CL-) that were allowed to feed on protein diet without cue lure exposure (Fig. 2). The sterile males exposed to yeast hydrolysate for 12-days period following cue lure feeding were more profoundly involved in lek initiation, lek participation and male calling. Cue lure feeding increased the attractiveness of protein fed males to females and increased the mating success by 2- and 3-times higher compare to P-CL- and P-CL+ males, respectively (Fig. 3). Exposure of 14-day old protein fed males to cue lure enhanced mating performance compare to the males from other treatments (Fig. 4). These results were supported by previous reports that ingestion of methyl eugenol and trimedlure increased the mating success through increased level of male signaling in *B. dorsalis* and *C. capitata* males 35. Sterile males from P+CL+ had highest FAI due to their increased attractiveness to their mates at all the ages tested. Females readily accepted 10 and 12-days old P+CL+ sterile males with FAI of nearly 0.6, followed by P+CL- males. Where as in P-CL+ males were least preferred by their mates with FAI ranged between 0.13-0.25 (Fig. 5).

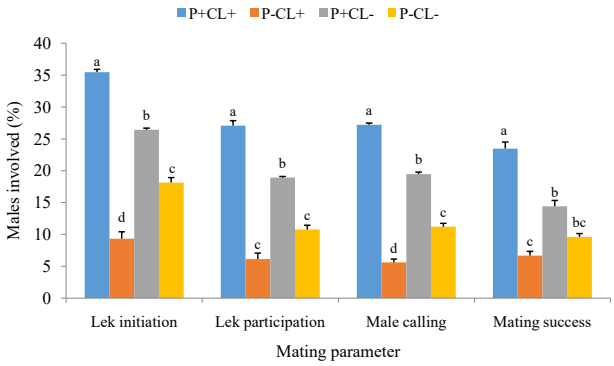


Fig. 2. Sexual performance of 10-days old sterile males exposed to dietary protein (P+/P-) and cue lure (CL+/CL-)

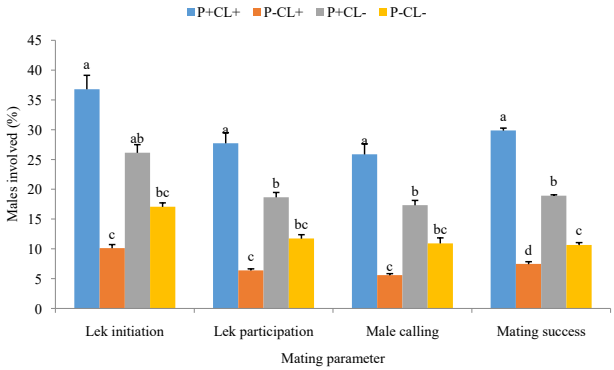


Fig. 3. Sexual performance of 12-days old sterile males exposed to dietary protein (P+/P-) and cue lure (CL+/CL-)

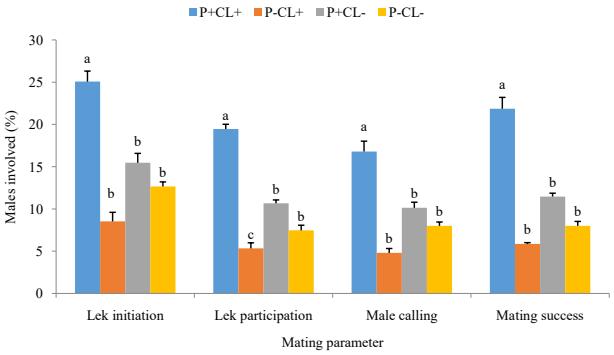


Fig. 4. Sexual performance of 14-days old sterile males exposed to dietary protein (P+/P-) and cue lure (CL+/CL-)

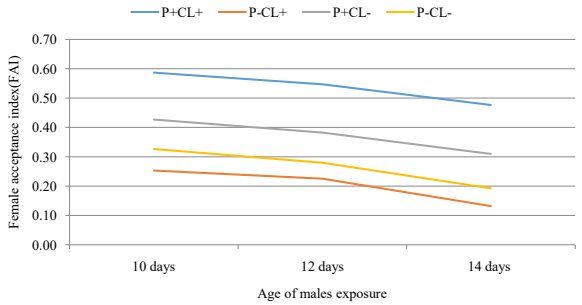


Fig. 5. Influence of adult dietary protein (P+/P-) and cue lure (CL+/CL-) on Female Acceptance Index of *B. cucurbitae* sterile males of different ages

Among the different age of males exposed, 10 and 12-days old P+CL+ males performed better in all the parameters tested as compared to 14-days old P+CL+ males. Exposure of cue lure to 12-days old protein fed males increased the lek initiation, lek participation, male calling and mating success by 28.0, 38.0, 47.0, and 34.0% over 14-days old P+CL+ sterile males. These results tally with the findings of Shelly and Villalobos (1995) that sterile males of 10-12 days old exposed to cue lure highly involved in male signalling and had high mating success on the day following exposure to cue lure. These results showed an additive effect of interaction of protein-rich diet and cue lure exposure on mating success of *B. cucurbitae.* Khoo and Tan (2000) that cue lure-fed males of *B. cucurbitae* attracted more females compared to males deprived of cue lure. The similar results were reported by Shelly et al. (1996) that the sterile *B. philippinensis* males exposed to methyl eugenol (ME) before release achieved more number of matings with females over wild males. In laboratory studies, it is also confirmed that exposure of P+ sterile males to ME+ increased the percentage of mating with wild females in *B. philippinensis* (Obra and Resilva, 2011). In all the age group exposed, sterile males fed on protein diet (P+CL-) had more matings than males fed only on sugar (P-CL-) or cue lure alone (P-CL+). Less percentage of P-CL+ males were involved in mating, compare to P-CL- males. Sterile males exposed to cue lure alone were least preferred by females for mating as cue lure treated males were not matured sexually due to lack of protein in their diet. It indicates that cue lure alone has adverse effect on FAI for sterile males in absence of protein in their pre-release diet. These results were on par with the findings of Orankanok et al. (2011) that sterile males fed on sugar–yeast hydrolysate combinations achieved significantly more matings than males fed only water in *B. dorsalis* and more matings than males fed only sugar, only yeast hydrolysate or only water in *B. correcta*. Recently, Haq et al. (2016) examined the effect of methyl eugenol treatment on the incidence of pairing in *B. dorsalis* and *B. carambolae* and found that Methyl eugenol feeding enhanced the competitiveness and increased the number of matings in *B. dorsalis*.

The positive influence of cue lure on sexual performance of *Z. cucurbitae* was short-lived compared to methoprene. Feeding of sterile males of *Z. cucurbitae* with protein-enriched diet up to 10-12 days of age and then exposure to cue lure enhanced their mating performance by increasing male signaling (male calling) and male attractiveness to females. The combinations of methoprene application and yeast hydrolysate-sugar diet showed an additive effect on increased mating success in *Z. cucurbitae* sterile males and a clear effect of methoprene exposure confirming the potential of this approach to improve operational SIT application against melon fly.

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AUTHOR CONTRIBUTION STATEMENT

KS and PGS conceived and designed research. PGS conducted experiments. SRK provided timely guidance in planning of the experiments. BP provided gamma radiation facilities.

CONFLICT OF INTEREST

No conflict of interest.

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REGULATION AND CHARACTERIZATION OF AMYLASE ENZYME SECRETION IN THE DIGESTIVE TRACT OF *ANTHERAEA ASSAMENSIS* HELFER

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ABSTRACT

***Antheraea assamensis* Helfer is an endemic non-mulberry lepidopteran species of North East India with high commercial demand for its golden hued silk. *Antheraea assamensis* suffers from a protozoan disease pebrine which lead to death of the silkworm. In this study, the effect of various factors (starvation, feeding, temperature, pH, Ca ion and larval stage) in the regulation of amylase secretion is compared in healthy and diseased muga silkworm. Moreover, enzyme zymography and purification of amylase from the midgut is also performed. Result shows that, the secretion of amylase in *Antheraea assamensis* in starved condition, significantly decreases. Refeeding experiments (after 2 days of starvation) suggest that amylase secretion sharply increases due to feeding stimulus. The purified enzyme molecular weight is confirmed as 56 kDa by SDS PAGE. The purification fold of purified enzyme is 34 times higher than the crude enzyme extract.**

Key words: *Antheraea assamensis*, pebrine disease, starvation, feeding, temperature, pH, Ca ion, larval stage, amylase activity, zymography, SDS PAGE, enzyme purification

Antheraea assamensis Helfer (*A. assamensis*) is one of the important non-mulberry Lepidopteran species which is a geographical indicator of Assam, India. It is reknown for producing magnificent golden huedmuga silk with high commercial demand. It is a semi domesticated, polyphagous insect which is primarily reared on *Persea bombycina* (Som) and *Litsea monopetala* (soalu) plant. (Tikader et al., 2013; Nath et al., 2013).The digestibility is mostly depends on enzyme amylase and (Tikader and Rajan, 2012). The ability of digestion lead to the growth, development and survival of the insect. In insects, Alpha-amylases (EC 3.2.1.1) catalyzes the hydrolysis of alpha-1,4 glycosidic bonds of starch and glycogen which further produced final products of maltose, maltotriose, and branched maltodextrins (Franco et al., 2000; Da Lage, 2018) followed by hydrolized to glucose by α-glucosidase(s) (Rahimi and Bandani, 2014). Activity of amylase has been investigated in different orders of insects (Hemmati et al., 2022; El-Didamony et al., 2022; Hori, 1970; Terra et al., 1988; Ferreira and Terra, 1989; Schumaker et al., 1993; Nagaraju and Abraham, 1995). In Lepidoptera, there are several reports showing the amylase enzyme activity in mulberry group (Yan et al., 2021; Yan et al., 2022; Abraham et al., 1992) but reports are less in non-mulberry silkworm.

Antheraea assamensis is often tainted with an intracellular parasite *Nosema assamensis* which causes the fatal disease pebrine. Pebrine infection in *A. assamensis* larva shows black spots in the whole body due to the melanin formation in the infected hypodermal cells. The larvae also become lazy, eat less compared to the healthy ones and growth decreases (Ganga, 2003). Similar symptoms were also reported in *Antheraea mylitta,* infected with protozoa *Nosema mylitta*. The larva showed extended development period, significant weight loss of infected larva and decrease in digestive enzyme secretion (Rath et al., 2003). Moreover, reduced digestive enzyme activity was also observed in young bees infected with *Nosema apis* (Malone et al., 1998). Therefore, this disease might be considered as an important factor which modulate the enzyme secretion in insects. Apart from disease, the other factors like feeding stimulus, temperature, pH, Ca ion etc. also modulate the secretion of enzymes from the endothelial cells of gut of insects. The activity and structural integrity of insect amylases are depend on Ca ion (Kaur et al., 2014). Starvation showed reduced synthesis of amylase leading to the lower secretion of amylase in *Acheta domesticus* (Teo and Woodring, 1985). Insect gut varies from acidic to alkaline in nature and therefore the secretion of digestive enzymes in the gut might depends on the pH. However, the role of these factors are less understood due to unavailability of investigation works. Nagaraju and Abraham (1995)

purified and characterized amylase enzyme from *Antheraea mylitta* but there is no report of amylase purification and characterization in other species of genus *Antheraea*, especially muga silkworm. Keeping these things in mind, the primary objective of this study is to determine the role of different factors like starvation and feeding, temperature, pH, Ca^{2+}, and developmental stages in crude amylase activity in the digestive tract of *A. assamensis* and to compare with pebrine infected silkworm. The study also aims for the purification and characterization of digestive amylase of *A. assamensis.*

MATERIALS AND METHODS

The eggs of prevalent semi domesticated stock (healthy) silkworms were collected from several parts of *A. assamensis* rearing areas of Assam ($25^0$52´N $91^0$14´E, $26^0$02´N$90^0$51´E, $26^0$27´N $92^0$02´E, $26^0$45´N $92^0$06´E) of Assam-Hahim, Kushdhoa, Mangaldoi odalguri, and Sapkhaity and Tura and Adakgiri of Meghalaya ($25^0$30´N$90^0$16´E, $25^0$15´N $91^0$23´E). Pebrine infected (diseased) silkworms were collected from Mangaldoi Seri culture farm, Assam. Otherwise all others were of green morphs. The study was conducted in the *Persea bombycina* garden of IASST, Paschim Boragaon, Guwahati, Assam. The eggs were incubated at room temperature and after hatching they were transferred to the Som trees. Experiments were set up in the wild condition and both the healthy stock and diseased larva were reared separately.

The gut of *A. assamensis* was dissected out and removed gut was rinsed three times in 1 X PBS, pH 7.4 and divided into foregut, midgut and hindgut. The gut sections were stored in 0.1 M sodium acetate buffer (pH 5.6) at -20°C (Blakemore et al., 1995). For the preparation of crude enzyme, the stored gut sections were homogenized in 500 µl of 0.1 M sodium acetate buffer (pH 5.6) on ice (Zeng et al., 2012). The opened gut tissues were centrifuged at 2000g for 2 minutes at 4^0 C. To test enzyme activity in tissue of different The tissue homogenate (TH) of individual healthy and diseased larvae were centrifuged at high speed (16,000 × *g*) for 10 min at 4^0 C separately and supernatant was subsequently used for enzyme assay. The control was prepared by same volume of 0.1 M sodium acetate buffer for both healthy and diseased larvae. Amylase activity was determined by the method adopted by Bernfeld, (1955) with minor modifications. The regulation of secretion of amylase of *A. assamensis* was determined by starvation and feeding assay by the method of Weidlich et al., (2013). For control groups, 5thinstar larvae for healthy and diseased silkworms were starved for 2 days and the guts were dissected. For refed groups, 5th instar larvae after 2 days of starvation feed them for the next 24 hours with *Persea bombycina* leaves and gut were removed. The optimum temperature and pH for amylase activity was performed by DNS method (Ma et al., 2014). The effect of Ca ion on amylase secretion was determined in both healthy and diseased groups (Weidlich et al., 2013; Ma et al., 2014).

Zymography for amylase activity was carried out according to the method of García-Carreño et al., (1993). The SDS Page gel was prepared by the method of Laemmli, (1970) and Kotkar et al., (2009) with minor alterations. For purification, initially from the larval midgut of *A. assamensis* crude extract was prepared (Zeng et al., 2012). Then the extract was precipitated with ammonium sulfate (30% and 70%) at 4 °C. Followed by centrifugation at 6000 g for 15 min, then 5mL of universal buffer was applied for dilution and dialyzed overnight at 4 °C. A Sepharyl G-100 column was run at a flow rate of 0.5mL/ min by applying the dialyzed sample equilibrated with universal buffer (Zibaee et al., 2012). Protein concentration of all the purified samples were measured by the method of Lowry et al. (1951). All the experiments were performed in 3 replications and results are considered as mean ± standard deviation (n=30). Standard deviations and all the graphical presentations were done using Origin version 6.1. Means were compared by using ANOVA (significance at $p<0.05$ and $p<0.01$) with individual Post Hoc Tukey analysis by SPSS version 18 software package (www.winwrap.com).

RESULTS AND DISCUSSION

Current study reported the activity and regulation of amylase in the digestive tract of *A. assamensis*. The present study is the first report on regulation of amylase enzyme in different parts of the gut of *A. assamensis* and compared with diseased silkworm. Results showed more amylase activity the all three parts of the gut of healthy *A. assamensis* as compared with the diseased larvae ($p<0.01$) and in both cases the mid gut showed highest activity ($p<0.01$, Fig. 1). Similar result was also observed in *Antheraea mylitta* where amylase secretion was decreased due to infection that alter their digestion process leading to impaired growth. (Rath et al., 2003; Madhusudhan et al., 2018).

In both healthy and diseased silkworm, refed larvae of 24 h showed highly significant result as compared to the 2 days starved 5th instar larvae ($p<0.01$) except

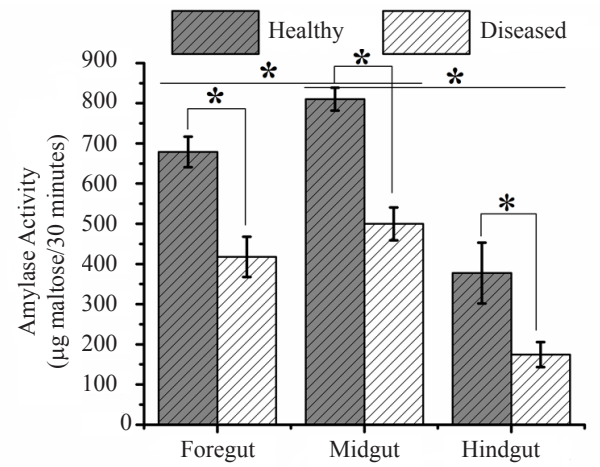


Fig. 1. Amylase activity in the different parts of the gut of healthy silkworms compared with diseased silkworms. Data showed significant difference among the groups of foregut, midgut and hindgut, ANOVA with post hoc tuckey**p<0.01 level of significance.

the hindgut of diseased ones. Prsent study findings indicated feeding as an important parameter for amylase activity. Here, the sharp increase in amylase activity after refeeding suggests that the presence of food content might act as a stimulus which increase amylase action (Fig. 2). It was also earlier reported that the presence of food in the gut acts as a prandial response which regulate the control release of digestive enzymes (Chapman, 1985; Lehane et al., 1995). Similar kind of result was also observed in *Gryllus bimaculatus* where the secretion of amylase is increased shortly

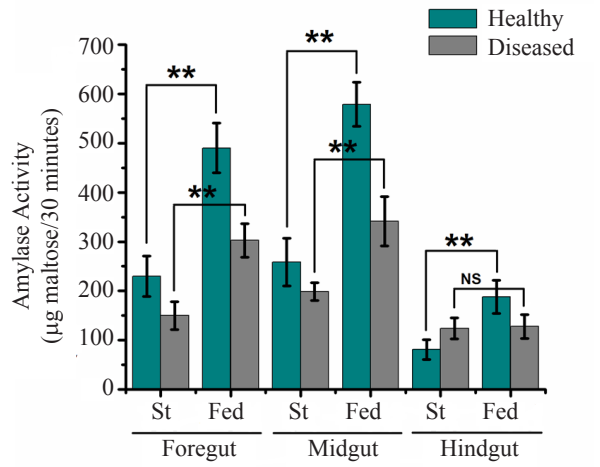


Fig. 2. Amylase activity in the different parts of the gut of healthy and diseased silkworm. In Refeeding experiment silkworms were starved for 2days and again reefed for 24 hours to observe amylase enzyme activity Data are significantly different among different groups of feed and starved, ANOVA with post hoc tuckey**p<0.01 level of significance.In the hind gut of amylase activity of infected larve showed no significant difference. Here St- Starved, Fed- feeding.

after molting as the larvae become voracious eater and consume increase amount of food (Weidlich et al., 2013) and in *Tribolium castaneum* (dos Santos et al., 2022). Likewise in lepidoptera also, with the advancement of developmental stages of different instars amylase activity is also increased (Nakonieczny et al., 2006; Willis et al., 2010). As the substrate is more, the enzyme activity is also more (Zadeh et al., 2020) and unavailability of substrate decreased or shut down the enzyme activity. This statement can be further supported with starvation experiment where the starved *A. assamensis* larvae showed less enzyme activity in comparison to the feeded ones. The food stimulus induce the secretion of neuropeptides which are released into the haemolymph and triggers the secretion of digestive enzymes (Sakai et al., 2006; Woodring et al., 2009; Mikani et al., 2011).

Results showed that amylase activity was highest at 35^0 C for both healthy and diseased larvae (p<0.01, Fig. 3 A). The optimum pH for the amylase activity was peak at pH 9 for both healthy and diseased larvae (p < 0.01, Fig. 3 B).The optimal temperature for amylase activity in insects is reported in the range of 30–60^0 C (Nadaf et al., 2022, Syakuro et al., 2022; Sarkar and Sikdar 2020; Kaur et al., 2014; Sharifloo et al., 2016) and in this regard, the present finding in *A. assamensis* also showed the same range in both healthy and diseased silkworm. *Antheraea mylitta* also showed highest activity of digestive amylase in gut at pH 9-10 (Nagaraju and Abraham, 1995) and in non-lepidoptera, *Rhynchophorus ferrugineus* and in *Eristalis megacephala* showed highest activity at pH 7-8 (Abd El-latif, 2020; Mohamed et al., 2019). The present study is in agreement with the observation that the high pH optima for digestive enzymes is the characteristics of polyphagous lepidopteran larvae (Syakuro et al., 2022, Nadaf et al., 2022, Zibaee et al., 2012).

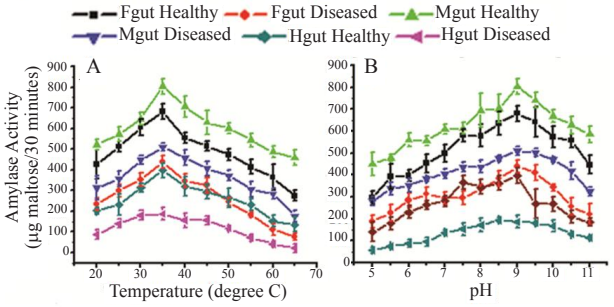


Fig. 3. Activity of amylase in respect to in a range of temperature (A) and pH (B) for both healthy and diseased silkworm. Data in terms of mean±sd, ANOVA with post hoc tuckeyp<0.01 level of significance.

The present investigation attempts to find out the mechanism of enzyme secretion (either apocrine or exocytosis mode) in both healthy and diseased muga silkworm. Current study showed an inhibitory effect of calcium ions on amylase activity which indicates that Ca ion might play a role in amylase secretion (Fig. 4 A, B). The possible mechanism of amylase secretion in the gut of *A. assamensis* is apocrine secretion instead of exocytosis as reported by several researchers in different insects (Cristofoletti et al., 2001; Weidlich et al., 2013).

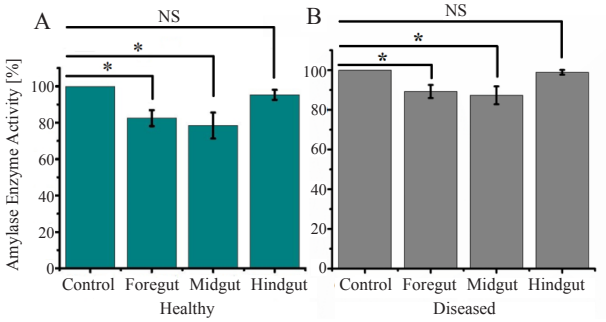


Fig. 4. Effect of calcium ion on amylase activity in the fore gut, mid gut and hind gut of healthy and diseased silkworm. Control hasn't incubated with calcium ions and converted to percentage enzyme activity, ANOVA with post hoc tuckey *p<0.05 level of significance and NS- not significant

The secretion of amylase had been significantly increased from 3rd instar to 5th instar stage in both healthy and diseased larvae (p<0.01, Fig. 5 A, B and C). The various developmental stages of insects act as one of the important factor in regulating enzyme activity (Weidlich et al., 2013). When *A. assamensis* silkworm emerges out from the eggs they start feeding with the tender leaves and after each molting they feed more. This implies that with the advancement of their age (1st, 2nd, 3rd, 4th, 5th instar) their food consumption rate is high and subsequently digestive enzymes activity also increase in both healthy and diseased silkworm. Study on amylase activity in *G. bimaculatus* also indicated high activity of enzyme in the last instar stage due to its high intake of food (Weidlich et al., 2013). Moreover, in current study the presence of amylase enzyme in gut of *A. assamensis* was confirmed by zymography. The mid gut of both diseased and healthy silkworm showed four similar peptide bands which indicated the presence amylase enzyme activity in both groups. However, the bands in diseased silkworm were faint in comparison to healthy one which indicated less amylase activity. Therefore, it might be stated that due to pebrine infection although, there was no change in the amylase enzyme but the overall amylase activity reduced.

A series of purification steps lead to isolation of a molecule with recovery of 18.72%, specific activity of 19.72 U/mg and purification fold of 34. Electrophoresis by SDS page followed by amylolytic activity revealed a molecular weight of 56 kDa for amylase enzyme of *A. assamensis*. (Table 1, Fig. 6). Ferreira and Terra, 1989 reported that in many insect the molecular weight of amylase ranges from 28 to 87 kDa, however in *Orius insidiosus* and *L. longipalpis*the molecular weight of

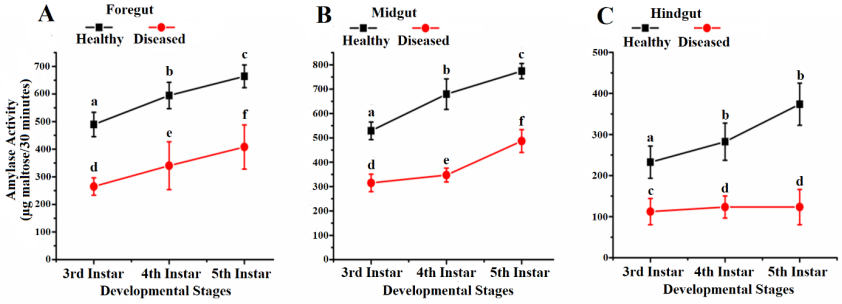


Fig. 5. Amylase in the gut of healthy/ diseased larvae -3rd instar to 5th instar

Table 1. Purification of amylase enzyme by different steps showing unit activity, specific activity, recovery percentage and purification folds

Purification steps	Unit activity (U± SE)	Amount of Protein (mg)	Specific Activity (U/mg Protein ± SE)	Recovery (%)	Purification fold
Crude extract	26.33± 0.57	45	0.58± 0.012	100	1
Ammonium sulfate (30%)	10.83± 0.28	20	0.54± 0.014	41.13	0.93
Ammonium sulfate (70%)	8.83± 0.15	6	1.47± 0.025	33.53	2.53
SepharylG-100	6.6± 0.173	0.45	14.66± 0.37	25.06	25.27
DEAE-Cellulose	4.93± 0.11	0.25	19.72± 0.44	18.72	34

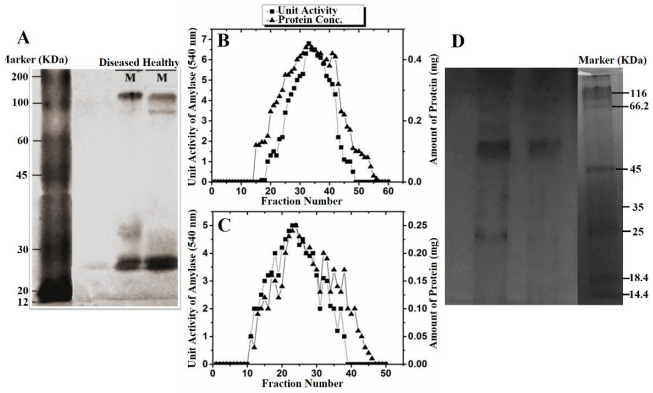


Fig. 6. Protein concentration of crude extract purified proteins in healthy and diseased larvae

amylase were 132 and 103 kDa respectively (Zeng and Cohen, 2000; Vale et al., 2012). A recent molecular study in Bark Beetle *Dendroctonus rhizophagus* (Curculionidae: Scolytinae) revealed the molecular weight of amylase was 53 kDa (Soto-Robles et al., 2021. In Lepidoptera, molecular weight of amylase was recorded as 51.2, 55 kDa for *Helicoverpa armigera* Hubner (Bhide et al., 2015), for *P. gossypiella* amylases was around 30 to 294 kDa (Nadaf et al., 2022) and 58 kDa for *Antheraea mylitta* (Nagaraju and Abraham, 1995). Furthermore, the variation of molecular weights of amylase enzyme due to compatibility properties of physiological role, interaction with dietary components and also depends on isoforms of the enzyme (Sharifloo et al., 2016).

Current study showed an alteration of amylase activity in diseased silkworms compared to healthy ones, but further in depth investigation is needed to understand the mechanism and characterization of amylase for their potential biotechnological applications is needed.

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AUTHOR CONTRIBUTION STATEMENT

Moni Kankana Kalita: Conceptualization, methodology, supervision, reviewing and editing; Kishor Haloi: Investigation, methodology and writing and original draft preparation; Dipali Devi: Editing and reviewing.

CONFLICT OF INTEREST

No conflict of interest.

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ARTIFICIAL INTELLIGENCE FOR CLASSIFICATION AND DETECTION OF MAJOR INSECT PESTS OF BRINJAL

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ABSTRACT

The present study was carried out during rabi 2020-21 at the College of Horticulture, Venkataramannagudem, West Godavari, Andhra Pradesh. Detection and monitoring of insect pests through Artificial intelligence (AI) were conducted using the Python software through Keras and Tensorflow frame works and for this purpose CNN VGG-16 model was used. A total of 204 insect images were loaded and included in four datasets. All the datasets were resized to 224 _ 224 pixels. CNN VGG-16 model codes developed for automatic pest classification and detection of pest images were run through python language to retrieve the predicted output. In the pursuit of detecting insect classification an accuracy of 95-98% for 4 insect classes viz., brinjal shoot and fruit borer larvae and adults, *Epilachna* beetle grub and adults were predicted with F1 score of 0.89 which shows that the CNN (VGG) model is consistent in detecting the type of insect.

Key words: Machine learning (ML), Convolutional neural networks, python language, keras, tensor flow, insect pest, insect images, prediction models, insect boxing, insect monitoring

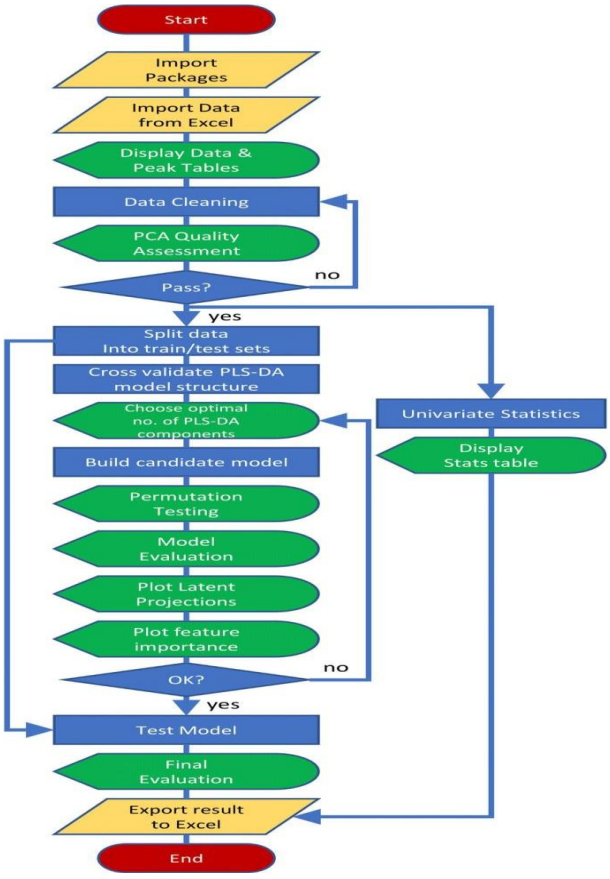
Artificial intelligence (AI) techniques provides the best possible solution for objective estimation of a large number of pest insects in a short time period through application of machine learning regression techniques at various stages of crop growth (Zhu et al., 2016) which can be an alternative to traditional way of pest monitoring and detection which requires long time for identification, classification, detection, counting and their damage assessment. In this paper we propose a new method to classify and detect insect pests based on Convolutional neural networks (CNN). The idea behind this method comes from the observation that the human eye does not focus on all its field of view, but accurately discriminates between its relevant and not relevant parts. Detection and monitoring of pests using AI is done through the Python software and other supporting frameworks such as Keras and Tensorflow by developing pest detection codes using VGG-16 model. The insect images in the pest datasets are subjected to bounding boxes (Kasinathan et al., 2020) to limit the size of the photo only to include the image of the insect. Further image augmentation techniques can be deployed to create new artificially generated image datasets for training and validation. The pre-trained model for pest classification and detection i.e., VGG Net (Garcia et al., 2020) can be used to extract the insect morphological features (feature extractor) which will distinguish the insect objects to classify unseen objects through codes / algorithm using python programming language. The training process of this VGG model will modify the input layer (Tuda et al., 2020) where the first few layers will be hidden in the dataset. The output layer will be dense and the fully connected layer with nodes where the output is visualized through Softmax activation function of the VGG model. The Artificial intelligence algorithm / codes that have been developed for insect pest classification and detection will predict accuracy to the original insect pest datasets. The invaluable information on early and quick detection of crop pests is currently being integrated into the pest management modules as a novel tool for pest identification, classification, monitoring, surveillance, prediction and timely interventions for pest suppression.

MATERIALS AND METHODS

In the present study AI / ML algorithm was used for classification and detection of insect pests in brinjal crop at various stages of crop growth. The data was analysed on 2.3 GHz Intel corei5 processor with 8 GB of RAM using the Python software and other supporting frameworks such as Keras and Tensorflow. A laptop with the configuration was utilized for this research viz., RAM of 8 GB and System Type of 64-bit Operating System, x64-based processor. The research

on detection and monitoring of insect pest classes of brinjal crop was executed on Anaconda platform of windows 10 along with Google drive for data storage. In the current analysis, the insect datasets were preprocessed, augmented, trained, tested and classified using Anaconda web-based platform applied by Google for implementing machine learning and deep learning models.. Tensor Flow and Keras are the primary libraries used herein and these packages were loaded onto the anaconda environment for model implementation. Total Power Unit (TPU) runtime available for free of cost in anaconda was utilized since the size of the data set used was around 8 GB. The insect pest data set acquired for this analysis was uploaded into google drive and then loaded into anaconda for further analysis. The primary reason for choosing google drive as a data repository for this analysis is that the size of the insect data set was around 4 GB. The CNN models for pest classification and detection was done through Python programming language where advanced importing packages such as Tensorflow and Keras were used for deep learning.

The various python libraries used for implementing CNN model to train the algorithm of insect datasets are NumPy, TensorFlow and Keras. Insect dataset for Insect classification and insect detection of brinjal crop was performed as suggested by Kasinathan et al. (2020). Insect Dataset had a total of 204 insect images of solanaceous and cruciferous crops. The total 204 insect images in the dataset were further grouped in to ten insect classes which were augmented for pest detection and monitoring. A total of 55 insect images were included per class, and they were divided into 80–20% train-test ratio. In dataset, the training set contained 163 insect images, and testing set contained 41 insect images. Image augmentation was applied for all the 204 insect images of brinjal crop mentioned in table 1. The insect images were rescaled to the size of 224_ 224 pixels. Image data augmentation techniques such as Horizontal and Vertical Shift Augmentation, Horizontal and Vertical Flip Augmentation, Random Rotation Augmentation and Random Zoom Augmentation were used to increase the training set for achieving improved accuracy and eliminate the problems of overtraining. Image augmentation was applied to insect dataset images to expand the training dataset. Later, shape features extracted from the insect images and ANN, SVM, KNN, and NB machine learning algorithms were applied to classify the insect classes. CNN based insect classification was adapted for comparison performance. The experiment was conducted with 9-fold cross-validation for insect dataset to evaluate the performance



Flow chart for insect pest detection algorithm (after Kasinatha et al., 2020)

of the machine learning algorithms. The flow chart as given here for pest detection algorithm was done using VGG-16 model (Kasinathan et al., 2020). Classification accuracy was calculated to compare the observed and the predicted outcome of insect pest dataset classes which revealed that the number of correct and incorrect predictions categorized. The classification accuracy of the VGG-16 model used for pest classification and detection was calculated as suggested by Kasinathan et al. (2020).

RESULTS AND DISCUSSION

The present study was carried out to classify and detect the insect pests viz**.,** brinjal shoot and fruit borer larvae and adults, *Epilachna* beetle larvae and adults in brinjal crop using AI/ML at various stages of crop growth. The different insect pest stage features were used for insect classification by applying ANN, SVM, KNN, NB, and CNN models. The performances of machine algorithms for two datasets were compared to provide insect class information and detection of insects performed with datasets. The insect pest detection algorithm is simple and efficient in terms of

computation time for detecting insects in agriculture fields. Image processing techniques were applied to segment the foreground of insect pests and locating the position of the insect in the image with a bounding box. Detection and monitoring of major pests of Brinjal crop through AI/ML were conducted on a 2.3 GHz Intel corei5 processor with 16 GB of RAM using the Python software and other supporting frameworks such as Keras and Tensorflow. The insect classification and detection were performed through image preprocessing, data augmentation, training and classification for various insect pests. The procedure for pest detection was done through AI / ML algorithm using VGG-16 model. A total of 204 insect images were loaded and resized to 224 _ 224 pixels. Open colour visualisation (CV) technique reads the color image in the order of BGR (Blue, Green, and Red) format.

CNN VGG-16 model Codes developed for automatic detection of 204 insect pest images given in four classes of data sets were run through python language. In the initial stage of study, images were acquired using the digital camera as suggested by Kasinathan et al. (2020). The size of the image was of the standard 1920 x 1080 and in SRGB color format. The pictures taken by digital camera contained a lot of noise in the form of fingers, leaves or the insects in the midst of other insects. We used bounding boxes method to limit the size of the photo only to include the image of the insect. We used image segmentation techniques using state of the art deep learning algorithms according to the procedure laid out by Liu et al. (2019) such as VGG to approximately isolate the shape of insect using the basis of a common insect. To improve the performance of the deep learning model, we have reduced the resolution to 50 x 50 to shorten the computational and training time and which also helpful to the farmers who usually use low-resolution cameras. Edge detection was used to mark out the edges of the insect since some of the insects were having same color such as the beetle adults and grubs. Initial results showed that edges detection gave erroneous results i.e., 2 or more insects were having similar shape but varied distinctly. Image processing techniques were applied to segment the foreground of insects and locating the position of all the insect images with a bounding box. Further image processing was done but it was time consuming and sometimes resulted in less accuracy than the model with no image processing applied, while it extracted feature in insects appearing visually distinct, in insects which were visually similar such as the adult beetle and the beetle grub, extraction of features made the images of both insects looksimilar, visually.

To increase the data sets for more accuracy, precision and F1 score, various image augmentation techniques were used viz., Horizontal and Vertical Shift Augmentation, Horizontal and Vertical Flip Augmentation, Random Rotation Augmentation and Random Zoom Augmentation. Using these processes, nine new artificially generated image datasets of the original insect pest dataset which was loaded for training were obtained. Further through this process of data augmentation, a total of 892 new images were produced with nearly 200 for each class. Out of the 892 new images produced by augmentation**,** The entire new dataset was divided in to 80:20 split ratio where 80% of the new augmented datasets were used for training and 10% was used for testing and the other 10% for validation of datasets. VGG model is the pre-trained convolutional neural network (CNN) invented by Simonyan and Zisserman from Visual Geometry Group (VGG) at University of Oxford in 2014 which is able to be the 1st runner-up of the ILSVRC (ImageNet Large Scale Visual Recognition Competition) 2014 in the classification task. VGG Net has been trained on ImageNet ILSVRC data sets which included images of 1000 classes split into three sets of 1.3 million training images, 100,000 testing images and 50,000 validation images. The model obtained 92.7% test accuracy in ImageNet. VGG Net has been successful in many real-world applications such as estimating the heart rate based on the body motion, and pavement distress detection.

VGG model was used with the purpose of enhancing classification accuracy by increasing the depth of the CNNs. VGG 16 and VGG 19, having 16 and 19 weight layers, respectively, was used for object recognition. VGG Net took input of 224 _224 RGB images and passed them through a stack of convolutional layers with the fixed filter size of 3×3 and the stride of 1. There were five max pooling filters embedded between convolutional layers in order to down-sample the input representation (image, hidden-layer output matrix, etc.). The stacks of convolutional layers were followed by 3 fully connected layers, having 4096, 4096 and 1000 channels, respectively. The pre trained model for insect pest classification and detection i.e,. VGG Net had extracted the insect morphological features (feature extractor) that can distinguish the objects and was used to classify unseen objects through codes / algorithm developed by using

python programming language. In the pursuit of detecting insect classification accuracy, CNN (VGG) model algorithm analysed through python software had an accuracy of 95-98% using 4 insect classes and 92-95% using 10 insect class datasets. The average accuracy metric showed that the present model accurately detect the type of insect across the dataset and F1 score shows perfect precision. The investigated model had an F1 score of 0.89 which signifies that the model is consistent in detecting the type of insect. The VGG model with multiple Convolutional Layers has the required power to identify patterns on the backs of insects. Regular Dropout layers will give us the way to train the model on unseen data using regular bitmasks. However, there was a subtle misclassification in the output pertaining to detection of beetle subclass viz., "epilachna beetle adults" and "epilachna beetle grubs" since all of these insect pests were grouped in single class by the VGG algorithm under the same feature. Overall, our CNN (VGG) model developed for pest classification and detection performed well with 95% accuracy to the original insect pest data.

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AUTHOR CONTRIBUTION STSTEMENT

NSK carried out the experiment, acquisition of images and analysed the data, and wrote the manuscript. NE supervised the experiments, provided technical support, and critically revised the manuscript. CH and KUK susbstantively revised it. All authors read and approved the final manuscript.

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COFLICT OF INTEREST

No conflict of interest.

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DISPERSAL OF LARVAE OF FALL ARMY WORM *SPODOPTERA FRUGIPERDA* IN MAIZE

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ABSTRACT

A comprehensive understanding of the pattern of larval dispersal is important to establish the criteria for sampling, statistical analysis and the development of reliable and sustainable management strategies. The fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), a notorious pest of maize all over the world was reported from the Indian subcontinent in 2018. The present study assessed plant-to-plant dispersal pattern of larvae of *S. frugiperda* in caged field conditions of maize. The mean distance travelled by the larvae after infestation ranged from 0.36± 0.36 to 1.05± 0.18 m. The maximum distance travelled by the larvae was up to 1.50 m. There was no significant difference between the number of larvae recovered concerning the direction (n-s) within the row. The distances covered by them in both directions were not significantly different either. The larval dispersal pattern suggested non-directional movement of the caterpillars.

Key words: *Spodoptera frugiperda*, *Zea mays*, behaviour, larval dispersal, movement, infestation, mean distance, management, sampling, non-directional movement, cages, field, directional movement

The fall army worm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) is a polyphagous pest with a wide host range of > 353 host plants (Montezano et al., 2018). This pest is native to American continent predominantly affecting maize (*Zea mays* L.) (Sparks, 1979; Martin et al., 1980). In 2018, this species invaded the Indian subcontinent and was reported for the first time on maize in the state of Karnataka (Ganiger et al., 2018). Ever since, it has spread to nearly all the Asia-Pacific nations, including Australia and New Zealand (CABI, 2022; EPPO, 2022). It significantly reduces both quality and total grain yield by causing extensive damage to the maize crop (Kasoma et al., 2021). The larvae damage the crop by feeding on leaves remaining inside the whorls during the vegetative crop stage and in the reproductive stage they attack and feed on the cobs. The total loss can be estimated up to 58% under suitable conditions (Chimweta et al., 2020). It has been estimated that India's maize output would be reduced by 37,000-75,000 tonnes, even if 5-10% production losses in the maize growing areas (Suby et al., 2020). The reasons for successful colonizing ability of *S. frugiperda* can be associated with its high reproductive ability, short generation time and dispersal behaviour (Johnson, 1987; Huang et al., 2021). Dispersal is described as a movement away from an area of high population density causing the spreading of individuals (Price, 1997; Shaw, 2020). Insects disperse to find resources such as food, mates, and shelter in order to maximise their potential (Skendzic et al., 2021). In case of *S. frugiperda*, the adult moth lays eggs in masses on some plants, but the infestation can be seen in the entire field within a short period of its initial occurrence. This suggests that the larvae might disperse to neighboring plants after hatching from the egg. In lepidopterans, initially larval dispersal occurs through ballooning with the help of silken thread and wind, whereas as the size of the larva increases in the later stages, movement occurs through walking which may happen at any time (Zalucki et al., 2002). Knowledge regarding the dispersal pattern of a pest, especially the larval movement pattern can provide vital information regarding behavioural and ecological understanding of an insect species including potential infestation levels (Garcia et al., 2021). The present study aimed to understand the plant-to-plant movement of *S. frugiperda* in the maize field which is of utmost importance for establishing adequate sampling criteria, statistical analysis and for the development of more reliable, economic, and sustainable management strategies (Taylor, 1984; Fernandes et al., 2003; Garcia et al., 2021).

MATERIALS AND METHODS

Dispersal pattern of larvae of *S. frugiperda* was studied in the field condition in three separate maize

plots during summer season in 2020 at the University of Agricultural Sciences, Bengaluru, India. Sweet corn, variety "Sugarita" was taken with a plot size of 4 x 4 m with five rows of plants, each consisting of 13 plants. The plants were raised with a spacing of 60 cm x 30 cm. After the germination of seeds, each study area was covered with nylon mesh (120 mesh) to prevent the external natural infestation by the *S. frugiperda*. Two weeks after the germination, one plant in every plot was artificially infested with one day old egg mass (≈130 – 140 eggs/ egg mass) obtained from the laboratory-reared culture. Artificial infestation of *S. frugiperda* was carried out by attaching one egg mass to the central plant of the middle row in each plot. The hatching % in every egg mass was recorded two days after release. The plants were inspected at two days of intervals and the new infestation on neighboring plants were recorded. The presence of larvae was ascertained on the 14th day of egg infestation by opening the whorls of plants. This method was followed to minimize the damage to plant whorl while searching for the larvae at every two-days interval.

In each plot, plants were oriented to north-south in the same row and east-west across rows. The larval location for the artificially infested plant (plant attached with egg mass) was considered as 'zero.' The number of larvae, as well as the mean distance and maximum distance moved by the larvae was estimated by the distance of infested plants from the release point with respect to different quadrants: northwest (nw), northeast (ne), southwest (se) and southeast (se); within the row (north and south) and across the rows (east and west). The survival % of larvae was calculated based on the number of larvae recovered fourteen days after infestation. The number of recovered larvae in each quadrant, orientation, and axes with respect to the infested plant as well as the maximum distance travelled by them was determined. Results were tested for normality and homogeneity of variance.

RESULTS AND DISCUSSION

In the three plots, the mean number of eggs infested was 134.67± 2.40. The egg hatching % was 92.12± 1.18%. But we recorded a lower number of larvae on plants after 14 days of artificial egg infestation (11.00± 0.58). Based on the number of larvae recovered after the 14th day, the mean larval survival rate was 8.86± 0.38%. This may be related to the high mortality of lepidopteran caterpillars in early stages. Similarly, high mortality of *S. frugiperda* in the early instars has been reported resulting in mere 5.06% larval survival after 14 days of egg infestation (Pannuti et al., 2016). However, the survivorship pattern has been also observed in western bean cutworm, *Striacosta albicosta* (Smith) in maize wherein a few larvae could stay alive to their maturity despite high egg survival (Paula-Moraes et al., 2013). In general, greater mortality at the early stage of their growth is typically seen in lepidopteran caterpillars. The reasons for low survival of larvae could be abiotic factors and biotic factors that governed mortality in early instars (Zalucki et al*.,* 2002). Most of the recovered larvae were found near to the egg infested plant in the same or adjacent row. Out of total larvae found in the field, 42.42± 0.67% of larvae were recovered within the same row as that of the egg infested plant and 48.48± 1.20% from the first adjacent row. A higher number of larvae (91%) from the nearby plants within the adjacent rows of the infested plant can be related to the food searching behaviour of the larvae. Upon hatching, the young larvae scrape green tissue for some time and then disperse to new plants to avoid competition and harvest food resource for better growth and development. The study from Pannuti et al. (2016) observed that *S. frugiperda* larvae were confined to within two rows from the plants which were artificially infested by the egg mass. A similar movement pattern of *S. frugiperda* was also observed in cotton where larvae travelled to 1 to 2.4 plants from the infested plant, not moving more than five plants from the release site (Ali et al., 1990). In maize cultivation, almost half of the *S. frugiperda* larvae recovered were from the infested row and 91.4% confined themselves within a radius of 1.1 m from the infested plant (Pannuti et al., 2016). Movement within the row is mostly predictable where more leaves are in close contact within a row than across rows (Blickenstaff, 1983; Ross and Ostlie, 1990).

In the present study, most of the larvae were recovered from the south direction within the infested row, but no significant difference was observed between north and south indicating that there is no specific directional movement of larvae, which corroborates with earlier findings by Pannuti et al. (2016). The number of newly infested plants on each alternate day after artificial egg infestation suggests that, greater proportion of healthy plants (more than 80%) got infested within 8 days of artificial infestation. The mean distance travelled by the larvae after infestation ranged from 0.36± 0.36 m to 1.05± 0.18 m. However, the maximum distance travelled by the larvae was up to 1.50 m on the 10th day after artificial infestation (Table 1). On the 14th day after the infestation of eggs, not more than one larva/ infested plant was recorded. The

Table 1. Larval dispersal of *S. frugiperda* in maize

Maximum distance travelled by larvae after artificially infested in caged conditions					
Days after artificial infestation	No. of newly infested plants		Mean distance travelled ± SE (m)	Maximum distance travelled (m)	
	Mean± SE	Proportion (%)			
2	2.67± 0.67	24.24	1.04± 0.04	1.34	
4	2.67± 0.88	24.24	0.64± 0.19	1.08	
6	2.33± 1.20	21.21	0.50± 0.26	1.24	
8	1.33± 0.33	12.12	1.05± 0.18	1.34	
10	1.67± 1.20	15.15	0.36± 0.36	1.5	
12	0.33± 0.33	3.03	0.45± 0.45	1.34	
			NS (p= 0.3802)		
Movement of larvae in different directions					
Direction	No. of infested plants	Total No. of larvae recovered		Mean distance travelled (m)	Maximum distance (m)
	Mean± SE	Mean± SE	Proportion (%)	Mean± SE	
Within quadrants					
ne	0.67± 0.67	0.67± 0.67	11.11	0.25± 0.25	0.85
nw	1.33± 0.88	1.33± 0.88	22.22	0.59± 0.32	1.34
se	2.00± 1.00	2.00± 1.00	33.33	0.75± 0.41	1.5
sw	2.00± 1.00	2.00± 1.00	33.33	0.78± 0.39	1.34
p value	NS (p= 0.69)	NS (p= 0.69)		NS (p=0.70)	
Within rows (Spacing between the plants 0.3 m)					
n	1.33± 0.33	1.33± 0.33	33.33	0.55± 0.05	0.6
s	2.67± 0.88	2.67± 0.88	66.67	0.72± 0.22	1.5
p value	NS (p= 0.23)	NS (p= 0.23)		NS (p= 0.50)	
Across rows (0.6 m between the rows)					
e	0.00± 0.00	0.00± 0.00	0	0.00± 0.00	0
w	0.33± 0.33	0.33± 0.33	100	0.20± 0.20	0.6
p value	NS (p= 0.37)	NS (p= 0.37)		NS (p= 0.37)	

NS= Non-significant @ p≥ 0.05; SE= Standard error

mean number of larvae recovered in each quadrant viz., ne, nw, se, se varied from 0.67± 0.67 to 2± 1.00. With respect to the quadrants, the mean distance moved by the larvae varied from 0.25± 0.25 m (ne) to 0.78± 0.39 m (sw). Similarly, the maximum distance travelled by the larvae varied from 0.85 (ne) to 1.50 m (se). There was no significant difference between the number of larvae recovered, the mean distance and the maximum distance moved by the larvae between the four quadrants (Table 1). The larval movement along and across the axes showed that a greater number of larvae moved towards the south within the infested row (n-s) with a recovery frequency of 66.67% and a maximum distance movement of 1.50 m from the infested plant. The mean distance moved by the larvae varied from 0.55± 0.05 m to 0.72± 0.22 m. There was no significant difference between the number of larvae recovered with respect to the direction (north-south) within the row. The distances moved by them in both the directions within the row were also on par. Regarding the larval movement across the infested row (e-w), only one larva was recovered from the west axis, whereas no larvae were recovered from the east axis. There was no significant difference between the number of larvae recovered and the distance travelled across the rows. These results indicate the non-directional movement of the larvae in the field.

In general, the larval movement varies from plant to plant and depends on type of crop and cultivation practices (Tavares et al., 2021). Active movement from one plant to another within or between rows (within or between resources) is correlated with a form of searching behaviour of insects which is also controlled by several factors viz., internal factors, external

environmental factors, and biological factors like the ability to perceive sensory information (Bell, 1990). This sensory information can also be non-directional (Barrows, 1975; Bell, 1990) or directional (Baker, 1978; Harris and Miller, 1984). Conversely, information from biological resources like a gradient of pheromone or food odour or sound or direct visual stimuli can be well localised (Baker, 1978; Bell, 1990). This kind of awareness enables the insect to monitor its path towards the biotic source of information (Bell, 1990) which may be correlated with the current finding of non-directional movement of *S. frugiperda* larvae. Further, the dispersal of *S. frugiperda* larvae mainly occurs by ballooning and crawling. This dispersal behaviour can be helpful in incorporating non-host crops or trap crops in crop cultivation practices (Kumar et al., 2022) as well as in spot application of insecticides (Li et al., 2023) in order to manage the notorious pest in a sustainable manner.

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AUTHOR CONTRIBUTION STATEMENT

MK conceived and designed the research. RB conducted the experiments and analysed the data. RB wrote the manuscript and both authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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SURVIVAL AND DEVELOPMENT OF *HELICOVERPA ARMIGERA* (HUBNER) ON PUBLIC SECTOR *BT* COTTON HYBRIDS

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ABSTRACT

Laboratory experiment was conducted to find out the survival and development of *Helicoverpa armigera* (Hubner) on public sector *Bt* cotton hybrids (NHH-44 BG II, PKV Hy-2 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II and G. COT-08 Hy. BG II). Instarwise observations were documented on leaves, squares, young bolls and mature bolls at 60-80, 90-110, 120-140 and 150-170 days old crop, respectively. The results revealed that the early instars of *H. armigera* exhibited higher larval mortality than the later instars. The surviving larvae revealed adverse effect on many parameters viz., larval and pupal weight, % pupation and adult emergence indicating low growth and survival indices.

Key words: *Helicoverpa armigera*, Bt cotton, hybrids, adult emergence, diet, bioassay, larval weight, pupal weight, pupation, emergence, growth, development

India is a leading cotton (*Gossypium* sp.) grower but its productivity is very low due to the influence of various abiotic and biotic factors. Biotic factors include insect pests, diseases and weeds. Of these, insect pests cause maximum yield loss, as right from germination to the final picking, the crop is subjected to severe damage by 162 species of insect pests (Manjunath, 2004). American bollworm *Helicoverpa armigera* (Hubner) is the major pest in Maharashtra (Bhamare and Wadnerkar, 2018), and cotton producing countries face havoc of such insect pest despite intensive pesticide use. Transgenic cotton technologies were commercialized in 1996 and these have changed the crop protection scenario, and Bt cottons incorporating *Bt*-toxins are now available (Kranthi, 2020). Recently many public sector Bt cotton hybrids are developed in India. In Maharashtra, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani and Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola have developed NHH-44 BG II and PKV Hy-2 BG II cotton hybrids, respectively. For Bt cotton technology, it is crucial to express cry toxin proteins in adequate quantity in appropriate plant parts (Bhullar and Gill, 2015). Investigations conducted in India indicate that Cry toxin concentration in Bt cotton transmutes with progressing season, as a result efficacy deviates among the hybrids at different plant age and in different plant structures (Rupnar, 2018; Likhitha and Bhamare, 2018; Kranthi et al., 2005). In view of introduction of public sector Bt cotton hybrids for farmers in the open market, the present study assesses the survival and development of *H. armigera* on different plant parts at different growth stages.

MATERIALS AND METHODS

The present study was conducted at the Post Graduate Laboratory, Department of Agricultural Entomology, College of Agriculture, Latur (Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani), Maharashtra during 2019-20. The public sector Bt cotton hybrids (NHH-44 BG II, PKV Hy-2 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II, G. COT-08 Hy. BG II and NHH-44 non-*Bt* as control) were cultivated in the Experimental Farm of Department of Agricultural Entomology following the recommended package of practices except plant protection. The initial culture of *H. armigera* was obtained by collecting large sized larvae from the surrounding fields of College of Agriculture, Latur. These were reared with feeding on natural diet (leaves, squares, young bolls and mature bolls of non-Bt cotton) till pupation. The sexes were determined in pupal stages on the basis of distance between genital and anal apertures (viz., less in the case of male and more in the case of female). The freshly emerged adults were released (with proportion of 1:5 female to male) into standard oviposition cage having cotton swab dipped into 10% honey solution and a strip of cotton cloth toweling hung vertically as oviposition substrate. After hatching from eggs on the toweling, the neonate larvae were transferred separately into plastic vials to avoid cannibalism and fed on natural diet till pupation. The different instar larvae obtained were used for further investigations.

The laboratory studies were carried out in completely

Table 1. Mortality (%) of *H. armigera* larvae fed on different plant parts of public sector Bt cotton hybrids

S. No.	Treatments	On leaves (60-80 days old crop)					On squares (90-110 days old crop)					On young bolls (120-140 days old crop)					On mature bolls (150-170 days old crop)				
		I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar
T_1	NHH-44 BG II	100.00 (90.00)*	86.67 (68.59)	60.00 (50.77)	50.00 (45.00)	0.00 (0.00)	80.00 (63.43)	70.00 (56.79)	36.66 (37.26)	20.00 (26.57)	0.00 (0.00)	100.00 (90.00)	86.67 (68.59)	56.67 (48.83)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	80.00 (63.43)	50.00 (45.00)	0.00 (0.00)	0.00 (0.00)
T_2	PKV Hy-2 BG II	100.00 (90.00)	90.00 (71.57)	70.00 (56.79)	56.67 (48.83)	0.00 (0.00)	100.00 (90.00)	93.33 (75.03)	76.67 (61.12)	60.00 (50.77)	0.00 (0.00)	80.00 (63.43)	66.67 (54.74)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	76.67 (61.12)	63.33 (52.73)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)
T_3	PDKV-JKAL-116 BG II	90.00 (71.57)	76.67 (61.12)	40.00 (39.23)	40.00 (39.23)	0.00 (0.00)	86.67 (68.59)	73.33 (58.91)	40.00 (39.23)	30.00 (33.21)	0.00 (0.00)	90.00 (71.57)	83.33 (65.90)	50.00 (45.00)	0.00 (0.00)	0.00 (0.00)	73.33 (58.91)	53.33 (46.91)	10.00 (18.43)	0.00 (0.00)	0.00 (0.00)
T_4	G. COT-10 Hy. BG II	86.67 (68.59)	70.00 (56.79)	33.33 (35.26)	30.00 (33.21)	0.00 (0.00)	90.00 (71.57)	80.00 (63.43)	50.00 (45.00)	40.00 (39.23)	0.00 (0.00)	76.67 (61.12)	60.00 (50.77)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	80.00 (63.43)	66.67 (54.74)	33.33 (35.26)	0.00 (0.00)	0.00 (0.00)
T_5	G. COT-08 Hy. BG-II	76.67 (61.12)	66.67 (54.74)	30.00 (33.21)	20.00 (26.57)	0.00 (0.00)	100.00 (90.00)	86.67 (68.59)	66.67 (54.74)	53.33 (46.91)	0.00 (0.00)	83.33 (65.90)	76.67 (61.12)	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)	83.33 (65.90)	76.67 (61.12)	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)
T_6	NHH-44 (Non- *Bt*)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE (m) ±	0.19	0.23	0.13	0.13	-	0.13	0.23	0.23	0.13	-	0.19	0.27	0.13	-	-	0.23	0.27	0.13	-	-
	CD p=0.05)	0.58	0.71	0.41	0.41	-	0.41	0.71	0.71	0.41	-	0.58	0.82	0.41	-	-	0.71	0.82	0.41	-	-
	CV %	4.41	6.28	6.06	7.19	-	3.09	6.07	9.07	6.95	-	4.65	7.57	6.84	-	-	5.92	8.31	8.65	-	-

*Figures in parentheses angular transformed values

randomized design (CRD) with three replications using ten larvae per replication, on the field collected plant structures viz., leaves, squares, young bolls and mature bolls of public sector *Bt* cotton hybrids at pre-determined intervals of 60-80, 90-110, 120-140 and 150-170 days old crop, respectively. The plant structures were collected in labeled plastic bags. Collected samples were cleaned and placed individually in a plastic vial. Later laboratory reared different larval instar larvae of *H. armigera* were released on different cotton structures. The plant parts were replaced regularly with fresh *Bt* plant parts (same on which larvae fed) till pupation. The data on mortality of larvae were recorded separately for each instar by feeding them on different plant structures of public sector *Bt* cotton hybrids at pre-determined intervals. Weight of the surviving larvae was recorded after 24, 48 and 72 hr of exposure and weight of pupae was also recorded from each treatment. In addition, other parameters viz., % pupation and adult emergence were documented from the surviving larvae and pupae, respectively. The growth index and survival index were calculated on different treatments using the formulae given by Vennila et al. (2006). The data in respect of survival and development was statistically analyzed by ANOVA with the null hypothesis tested by 'F' test- at p=0.05 (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The laboratory bioassay revealed that the fifth instar *H. armigera* larvae fed on different plant parts of public sector *Bt* cotton hybrids and NHH-44 non-*Bt* cotton showed no mortality. However, among the different public sector *Bt* cotton hybrids, I, II, III and IV larval instars of *H. armigera* fed on leaves and squares of PKV Hy-2 BG II exhibited maximum % mortality at 60-80 and 90-110 days old crop, respectively. Where, minimum mortality was recorded on leaves of G. COT-08 Hy. BG II at 60-80 days old crop and on squares of NHH-44 BG II at 90-110 days old crop. Maximum mortality was observed when larvae were fed on young and mature bolls of NHH-44 BG II at 120-140 and 150-170 days old crop, respectively; and minimum % mortality of larval instars was on young bolls of G. COT-10 Hy. BG II at 120-140 days old crop and on mature bolls of PDKV-JKAL-116 BG II at 150-170 days old crop (Table 1). Similar trend was observed as regards reduction in larval weight of I, II, III, IV and V instars which survived beyond 24, 48 and 72 hr after exposure; decreasing % pupation and adult emergence, and reduced pupal weight were found to result in low growth and survival indices, when reared on leaves and squares of PKV Hy-2 BG II. Where, the young bolls and

Table 2. Effect on larval weight of *H. armigera* fed on different plant parts of public sector Bt cotton hybrids

S. No.	Treatments	Mean larval weight (mg per larva)														
		I instar			II instar			III instar			IV instar			V instar		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
	On leaves (60-80 days old crop)															
T_1	NHH-44 BG II	7.18	19.76	38.40	21.99	29.05	39.16	83.3	87.6	127.86	199.84	230.5	255.2	300.8	306.2	319.5
T_2	PKV Hy-2 BG II	6.94	17.74	35.23	19.41	26.86	36.5	80.53	86.06	114.16	187.3	224.8	250.27	285.36	290.43	309.86
T_3	PDKV-JKAL-116 BG II	7.74	20.33	39.41	23.53	32.23	42.46	87.63	90.26	145	210.2	253.5	279.47	303.16	319.6	323.43
T_4	G. COT-10 Hy. BG II	9.01	22.45	43.42	26.00	38.03	47.86	90.35	106.8	154.33	215.76	278.16	297.77	308.1	330.2	350.1
T_5	G. COT-08 Hy. BG-II	9.74	29.45	58.78	32.83	46.93	53.63	96.16	112.67	162.83	232.73	282.5	321.1	310.33	334.8	358.6
T_6	NHH-44 (Non- *Bt*)	15.33	38.27	76.95	46.56	72.87	86.96	108.7	156.33	192.26	243.63	295.86	379.53	324.83	380.76	416.46
	SE (m) ±	0.26	0.32	0.55	0.54	0.87	0.94	0.84	1.09	2.17	0.87	0.83	1.34	1.53	1.45	1.30
	CD (p=0.05)	0.81	1.00	1.67	1.63	2.65	2.85	2.56	3.32	6.58	2.63	2.52	4.06	4.66	4.39	3.96
	CV %	4.98	2.31	1.97	3.29	3.69	3.19	1.60	1.77	2.51	0.11	0.55	0.78	0.87	0.76	0.65
	Initial weight	4.00	-	-	14.61	-	-	64.61	-	-	179.68	-	-	268.18	-	-
	On squares (90-110 days old crop)															
T_1	NHH-44 BG II	7.9	28.87	54.22	32.29	37.76	58.53	94.6	104.23	160.36	237.53	281.74	317.4	297	320.83	353.7
T_2	PKV Hy-2 BG II	5.47	16.31	36.04	20.74	26.73	35.13	79.51	89.93	113.46	182.33	221.25	244.58	274.7	288.76	305.4
T_3	PDKV-JKAL-116 BG II	7.36	21.85	42.56	27.93	36.00	46.33	88.6	99.63	153.23	213.36	276.13	295.2	294.93	316.5	349.43
T_4	G. COT-10 Hy. BG II	6.25	19.57	39.95	24.36	28.5	39.53	86.86	95.56	143.33	208.06	249.43	277.83	286.7	306.03	320.63
T_5	G. COT-08 Hy. BG-II	6.04	18.51	39.76	22.36	25.56	37.23	81.88	92.46	125.2	197.9	228.13	253.23	281.26	299.43	317.43
T_6	NHH-44 (Non- *Bt*)	10.42	34.56	73.55	28.03	74.5	88.53	102.3	150.2	192.76	251.96	295.7	376.86	320.03	383.06	410.66
	SE (m) ±	0.18	0.38	1.06	0.71	0.87	0.98	1.00	1.23	0.82	1.38	1.66	1.27	1.64	1.25	1.64
	CD (p=0.05)	0.55	1.17	3.23	2.17	2.64	2.98	3.03	3.74	2.51	4.21	5.05	3.88	4.99	3.79	4.98
	CV %	4.38	2.88	3.87	4.78	3.94	3.35	1.95	2.03	0.96	1.11	1.11	0.75	0.97	0.67	0.83
	Initial weight	3.27	-	-	21.27	-	-	75.12	-	-	178.02	-	-	265.91	-	-
	On young bolls (120-140 days old crop)															
T_1	NHH-44 BG II	6.38	18.44	38.01	20.88	31.8	56.53	93.68	107.5	118.43	188.83	226.26	250.76	271.4	299.08	313.33
T_2	PKV Hy-2 BG II	9.94	24.99	44.31	32.23	52.2	69.66	112.02	118.43	156.13	232.33	281.23	298.53	316.16	335.66	360.16
T_3	PDKV-JKAL-116 BG II	7.18	20.71	41.15	28.63	37.4	59.56	98.26	110.53	130.13	201.43	232.86	255.8	286.83	303.6	319.93
T_4	G. COT-10 Hy. BG II	10.56	31.11	59.18	36.13	59.7	73.76	117.33	120.83	162.83	263.96	283.7	323.38	329.56	340.03	368.95
T_5	G. COT-08 Hy. BG-II	8.45	22.66	41.82	30.68	45.86	62.36	102.23	114.53	147.66	212.73	251.93	281.13	301.43	329.6	354.66
T_6	NHH-44 (Non- *Bt*)	12.08	35.61	78.21	44.6	80.5	88.5	121.36	165.63	201.16	276.16	299.46	375.46	337.46	374.03	414.46
	SE (m) ±	0.30	0.70	0.53	0.79	1.20	1.30	1.53	1.46	1.36	1.18	1.40	1.19	1.14	1.22	1.05
	CD (p=0.05)	0.91	2.13	1.61	2.40	3.64	3.96	4.66	4.43	4.13	3.60	4.27	3.63	3.45	3.72	3.20
	CV %	5.73	4.75	1.82	4.26	4.06	3.30	2.47	2.06	1.54	0.89	0.92	0.69	0.64	0.64	0.51
	Initial weight	4.22	-	-	25.08	-	-	81.23	-	-	184.68	-	-	268.68	-	-
	On mature bolls (150-170 days old crop)															
T_1	NHH-44 BG II	5.87	19.46	38.53	25.2	42.6	62.1	94.63	109.43	122.26	189.53	236.53	256.03	271.86	305.5	311.86
T_2	PKV Hy-2 BG II	8.95	25.53	53.38	32.5	52.46	74.73	115.1	119.91	162.2	247.26	283.76	316.2	332.6	339.1	367.86
T_3	PDKV-JKAL-116 BG II	11.09	31.58	58.75	36.9	57.46	88.96	120.36	122.46	175.83	271.1	288.46	337.13	336.83	342.56	385.5
T_4	G. COT-10 Hy. BG II	8.17	22.8	48.83	31.56	48.16	77.33	104.43	114.63	174.96	219.78	273.8	285.9	317.63	337.6	348.06
T_5	G. COT-08 Hy. BG-II	6.88	21.56	44.8	30.46	45.7	72.87	99.8	112.01	133.26	202.86	264.4	259.13	304.9	331.36	338.56
T_6	NHH-44 (Non- *Bt*)	14.11	35.98	72.73	42.16	74.73	94.16	128.5	164.63	203.26	279.16	300.4	375.16	366.56	388.56	435.86
	SE (m) ±	0.27	0.75	0.90	0.91	0.97	1.23	1.02	0.73	1.11	0.79	1.52	1.17	1.19	1.26	1.44
	CD (p=0.05)	0.82	2.29	2.73	2.78	2.97	3.75	3.10	2.23	3.39	2.40	4.61	3.55	3.62	3.82	4.39
	CV %	5.12	5.00	2.95	4.80	3.16	2.73	1.60	1.03	1.23	0.09	0.96	0.66	0.64	0.64	0.68
	Initial weight	3.08	-	-	25.70	-	-	85.67	-	-	191.35	-	-	272.41	-	-

Table 3. Pupation (%) of *H. armigera* larvae fed on different plant parts of public sector Bt cotton hybrids

S. No.	Treatments	On leaves (60-80 days old crop)					On squares (90-110 days old crop)					On young bolls (120-140 days old crop)					On mature bolls (150-170 days old crop)				
		I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar
T_1	NHH-44 BG II	00.00 (00.00)*	13.33 (21.41)	40.00 (39.23)	50.00 (45.00)	100.00 (90.00)	20.00 (26.57)	30.00 (33.21)	63.33 (52.73)	80.00 (63.43)	100.00 (90.00)	00.00 (00.00)	10.00 (18.43)	43.33 (41.17)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	20.00 (26.57)	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)
T_2	PKV Hy-2 BG II	00.00 (00.00)	10.00 (18.43)	30.00 (33.21)	43.33 (41.17)	100.00 (90.00)	00.00 (00.00)	6.67 (14.97)	23.33 (28.88)	40.00 (39.23)	100.00 (90.00)	20.00 (26.57)	33.33 (35.26)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	23.33 (28.88)	36.67 (37.27)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)
T_3	PDKV-JKAL-116 BG II	10.00 (18.43)	23.33 (28.88)	60.00 (50.77)	60.00 (50.77)	100.00 (90.00)	13.33 (21.41)	26.67 (31.09)	60.00 (50.77)	70.00 (56.79)	100.00 (90.00)	00.00 (00.00)	13.33 (21.41)	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)	26.67 (31.09)	46.67 (43.09)	90.00 (71.57)	100.00 (90.00)	100.00 (90.00)
T_4	G. COT-10 Hy. BG II	13.33 (21.41)	30.00 (33.21)	66.67 (54.74)	70.00 (56.79)	100.00 (90.00)	10.00 (18.43)	20.00 (26.57)	50.00 (45.00)	60.00 (50.77)	100.00 (90.00)	23.33 (28.88)	40.00 (39.23)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	20.00 (26.57)	33.33 (35.26)	66.66 (54.73)	100.00 (90.00)	100.00 (90.00)
T_5	G. COT-08 Hy. BG-II	23.33 (28.88)	33.33 (35.26)	70.00 (56.79)	80.00 (63.43)	100.00 (90.00)	00.00 (00.00)	13.33 (21.41)	33.33 (35.26)	46.67 (43.09)	100.00 (90.00)	16.67 (24.10)	30.00 (33.21)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)	16.67 (24.10)	23.33 (28.88)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)
T_6	NHH-44 (Non- *Bt*)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	SE (m) ±	0.19	0.23	0.13	0.13	-	0.13	0.23	0.23	0.13	-	0.19	0.27	0.13	-	-	0.23	0.27	0.13	-	-
	CD (p=0.05)	0.58	0.71	0.41	0.41	-	0.41	0.71	0.71	0.41	-	0.58	0.82	0.41	-	-	0.71	0.82	0.41	-	-
	CV %	13.63	11.66	3.85	3.50	-	9.86	12.45	7.42	3.56	-	11.76	12.47	3.59	-	-	13.12	10.87	3.23	-	-

* Figures in parentheses angular transformed values

Table 4. Pupal weight of surviving *H. armigera* larvae fed on different plant parts of public sector Bt cotton hybrids

S. No.	Treatments	On leaves (60-80 days old crop)					On squares (90-110 days old crop)					On young bolls (120-140 days old crop)					On mature bolls (150-170 days old crop)				
		I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V istar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar
T_1	NHH-44 BG II	00.00	152.56	176.23	189.86	303.93	166.6	167.16	184.83	194.26	353.70	00.00	152.23	169.70	277.16	280.66	00.00	162.66	175.80	278.53	305.50
T_2	PKV Hy-2 BG II	00.00	149.33	168.00	170.13	267.10	00.00	146.66	162.13	162.23	305.40	177.73	176.96	197.93	320.26	332.66	194.50	180.26	197.56	329.10	339.10
T_3	PDKV-JKAL-116 BG II	155.30	160.46	185.30	201.66	312.73	152.2	161.30	183.16	188.16	349.43	162.6	158.73	176.06	315.26	319.56	202.93	183.43	204.96	335.03	342.56
T_4	G. COT-10 Hy. BG II	166.26	168.03	194.23	210.43	322.83	151.9	156.46	176.30	176.68	320.63	184.90	185.40	204.20	330.93	341.33	187.66	176.56	191.80	323.10	337.60
T_5	G. COT-08 Hy. BG-II	173.40	174.96	201.63	217.96	328.40	00.00	155.43	166.66	167.13	317.43	164.53	164.06	186.73	323.16	324.03	184.13	166.70	187.63	317.80	331.36
T_6	NHH-44 (Non- *Bt*)	286.39	338.9	339.06	342.43	350.03	278.0	331.35	334.33	335.46	410.66	296.46	336.66	337.16	362.26	363.36	333.10	338.73	336.73	364.03	388.56
	SE (m) ±	0.60	1.65	0.87	0.07	1.44	0.77	1.08	0.96	0.97	1.64	1.18	0.91	0.84	1.04	1.13	0.87	1.03	0.74	1.12	1.26
	CD (p=0.05)	1.83	5.00	2.66	2.39	4.37	2.36	3.30	2.91	2.94	4.98	3.59	2.76	2.56	3.15	3.44	2.65	3.14	2.26	3.41	3.82
	CV %	0.80	1.49	0.72	0.61	0.79	1.08	1.01	0.82	0.82	0.83	1.25	0.80	0.69	0.56	0.60	0.82	0.89	0.59	0.60	0.64

Table 5. Adult emergence (%) from *H. armigera* larvae fed on different plant parts of public sector Bt cotton hybrids

S. No.	Treatments	On leaves (60-80 days old crop)					On squares (90-110 days old crop)					On young bolls (120-140 days old crop)					On mature bolls (150-170 days old crop)				
		I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar
T_1	NHH-44 BG II	0.00 (0.00)*	10.00 (18.43)	30.00 (33.21)	46.67 (43.09)	100.00 (90.00)	20.00 (26.57)	30.00 (33.21)	60.00 (50.77)	73.33 (58.91)	100.00 (90.00)	0.00 (0.00)	6.67 (14.97)	26.67 (31.09)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	10.00 (18.43)	43.33 (41.17)	100.00 (90.00)	100.00 (90.00)
T_2	PKV Hy-2 BG II	0.00 (0.00)	0.00 (0.00)	23.33 (28.88)	40.00 (39.23)	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)	13.33 (21.41)	33.33 (35.26)	100.00 (90.00)	20.00 (26.57)	26.67 (31.09)	63.33 (52.73)	100.00 (90.00)	100.00 (90.00)	13.33 (21.41)	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)
T_3	PDKV-JKAL-116 BG II	10.00 (18.43)	20.00 (26.57)	33.33 (35.26)	56.67 (48.83)	100.00 (90.00)	13.33 (21.41)	23.33 (28.88)	50.00 (45.00)	60.00 (50.77)	100.00 (90.00)	0.00 (0.00)	10.00 (18.43)	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	20.00 (26.57)	40.00 (39.23)	86.67 (68.59)	100.00 (90.00)	100.00 (90.00)
T_4	G. COT-10 Hy. BG II	13.33 (21.41)	30.00 (33.21)	50.00 (45.00)	66.67 (54.74)	100.00 (90.00)	10.00 (18.43)	20.00 (26.57)	43.33 (41.17)	53.33 (46.91)	100.00 (90.00)	23.33 (28.88)	40.00 (39.23)	66.67 (54.73)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	26.67 (31.09)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)
T_5	G. COT-08 Hy. BG-II	20.00 (26.57)	33.33 (35.26)	60.00 (50.77)	73.33 (58.91)	100.00 (90.00)	0.00 (0.00	10.00 (18.43)	20.00 (26.57)	46.67 (43.09)	100.00 (90.00)	10.00 (18.43)	20.00 (26.57)	53.33 (46.91)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	20.00 (26.57)	53.33 (46.91)	100.00 (90.00)	100.00 (90.00)
T_6	NHH-44 (Non- *Bt*)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	SE (m) ±	0.13	0.13	0.19	0.27	-	0.13	0.13	0.19	0.27	-	0.13	0.19	0.27	-	-	0.13	0.13	0.23	-	-
	CD (p=0.05)	0.41	0.41	0.58	0.82	-	0.41	0.41	0.58	0.82	-	0.41	0.58	0.82	-	-	0.41	0.41	0.71	-	-
	CV %	9.86	7.31	6.74	7.37	-	9.86	7.71	6.97	7.71	-	9.22	8.95	8.08	-	-	9.86	6.23	5.92	-	-

* Figures in parentheses angular transformed values

Table 6. Growth and survival indices of *H. armigera* reared on different plant parts of public sector *Bt* cotton hybrids

Treatments	Growth Index				Survival Index			
	Leaves	Squares	Young bolls	Mature bolls	Leaves	Squares	Young bolls	Mature bolls
NHH-44 BG II	0.75	1.30	0.88	0.94	0.38	0.56	0.46	0.50
PKV Hy-2 BG II	0.64	0.59	1.34	1.37	0.33	0.29	0.62	0.62
PDKV-JKAL-116 BG II	0.99	1.12	1.01	1.77	0.44	0.49	0.50	0.69
G. COT-10 Hy. BG II	1.16	0.94	1.48	1.25	0.52	0.45	0.66	0.59
G. COT-08 Hy. BG-II	1.36	0.72	1.20	1.11	0.58	0.35	0.56	0.54
NHH-44 (Non- *Bt*)	2.38	2.56	2.77	3.03	1.00	1.00	1.00	1.00

mature bolls of NHH-44 BG II when fed to different larval instars of *H. armigera* demonstrated reduction in same growth and developmental parameters (Tables 2-6).

The bioassay done with different plant parts of public sector *Bt* cotton hybrids conclusively elucidated decrease in mortality rate of *H. armigera* from early stage (leaves and squares) to the later stage (young and mature bolls) of crop. Thus, the expression of Cry toxins significantly declined with the progression of plant age. This view derives support from the earlier findings (Jayaprakash et al., 2013; Baoqian et al., 2011, Arshad et al. 2009, Siebert et al., 2009; Kranthi et al., 2005). The data also revealed higher larval mortality of early instars fed on different plant parts compared to the later instars. However, the surviving late instars showed conflicting effects on the growth and development, such as reduced larval weights, pupation pupal weight and adult emergence. Also, the growth and survival index values became low when reared on leaves and squares compared to young bolls and mature bolls. These results are in accordance with those of earlier workers (Rupnar, 2018; Hallad et al., 2011; Likhitha and Bhamare, 2018, Mahalakshmi and Prasad, 2013; Naik et al., 2012). Among the different public sector *Bt* cotton hybrids superior results were expressed by PKV Hy-2 BG II when *H. armigera* larval instars fed on leaves and squares at 60-80 and 90-110 days old crop, respectively and NHH-44 BG II when fed on young bolls and mature bolls at 120-140 and 150-170 days old crop, respectively.

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REPORT OF GUAVA FRUIT FLY *BACTROCERA CORRECTA* BEZZI FROM TEMPERATE KASHMIR

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ABSTRACT

In this study the guava fruit fly *Bactrocera correcta* Bezzi (1916) (Diptera: Tephritidae) is reported from Kulgam, a major peach growing area in South Kashmir through July-August, 2020. District Kulgam was the leading producer of peaches with an annual production of 412MT during the 2018-19 growing season. This potential pest was trapped along with *B. zonata* and *B. dorsalis* in a simple bottle-type trap containing methyl eugenol and SPLAT.

Key words: *Bactrocera correcta*, Tephritidae, parapheromone, methyl eugenol, SPLAT, infestation, simple-bottle traps, Kulgam, J&K, bottle type trap, trap catches

Bactrocera correcta (Bezzi, 1916) is an invasive pest in tropical and subtropical countries. In India, it was first reported in 1916 from Bihar (Bezzi, 1916). It was subsequently reported from other States such as Tamil Nadu, Karnataka, Gujrat, Madya Pradesh, Haryana, Himachal Pradesh, and Punjab. *Bactrocera correcta* infests >70 species of fruits in tropical and subtropical regions. In India, *B. correcta* is also considered a serious pest that causes almost 80% of fruit damage (Jalaluddin, et al., 1999). The main attractants used to capture *B. correcta* are methyl eugenol, ethyl dodecanoate, ethyl palmitate, ethyl oleate, tetradecanoate, and ethyl stearate (Jalaluddin, et al., 1999); Kawashita et al., 2004; Tokushima, et al., 2010). Males of *B. correcta* were attracted to a mixture of β-caryophyllene and α-humulene (Tan, 2010). In this study, district Kulgam of UT of Jammu and Kashmir where *B. correcta* has been reported is discussed herein in the background of maximum peach production of 412 mt (Ahmad, et al., 2021). There is a need to understand the spread and dispersal of *B. correcta* in Kashmir.

MATERIALS AND METHODS

A survey was conducted through 2019 and 2020, in 30 sites of fruit and vegetable growing areas of Kashmir valley. A total of 94 traps made from simple cold drink bottles covered with a yellow adhesive tape containing methyl eugenol inside hanging rubber septa and SPLAT at the bottom were used. Three equidistant holes (1.5 cm^2) were made with a hot T-type handle spanner as well as with a glue gun for the entry of fruit flies. Traps were set on 1st March 2019 and retrieved fortnightly (14-15 days later) from the date of installation to 17th November 2020, with a total of 18 visits/ site/ year. Only district Kulgam was surveyed with 6 traps (SKU1=03, SKU2=05, SKU3=08) were installed, and a total of five traps were installed- one each on peach (*Prunus persica*) (Rosaceae), Apple (*Malus pumilla*) (Rosaceae), Bottle gourd (*Lagenaria siceraria*) (Cucurbitaceae), Cucumber (*Cucumis sativus*) (Cucurbitaceae) and Quince (*Cydonia oblonga*) (Rosaceae), at the site SKU2 (Tarigam- 33.7081388°, 75.03809134°, 1569 masl). All the installed traps were checked and refreshed fortnightly, and the *Bactrocera* spp. adults collected were deposited at the Zoology Museum as voucher specimens. Samples were identified by consulting keys and for further confirmation by Prof Drew, (ICMF, Griffith University, Queensland, Australia). The result of each trap site was analyzed for the population index, which estimates the number of fruit flies trapped/ day (Trapping Guidelines for Area-Wide Fruit fly Programmes, 2003) as FTF = F/T x D. Where, FTF = fruit flies trapped per day, F = Total number of fruit flies trapped, T = Total number of serviced traps, D = Number of days traps exposed in the site.

RESULTS AND DISCUSSION

Bactrocera spp. were observed flying around and

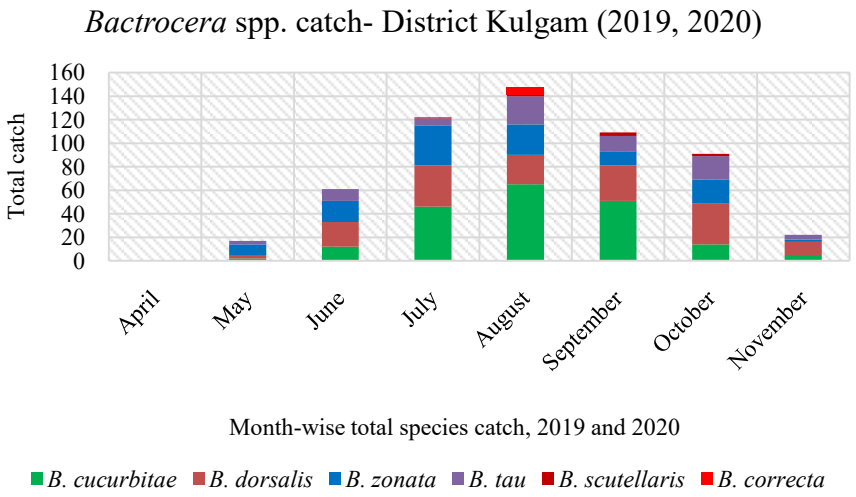


Fig. 1. *Bactrocera* spp. catches (District Kulgam, 2019 and 2020)

landing on *P. persica* and *C. sativus* plants. A total of six species, namely *B. cucurbitae, B. dorsalis, B. tau, B. scutellaris,* and *B. zonata,* were captured from almost all 30 locations and *B. correcta* has been detected in August, 2020 in district Kulgam of South Kashmir. *Bactrocera correcta* collected were studies under a Leica S9D+ stereomicroscope with MC170HD camera. A total of 6 adult males were trapped in August, of the 569 fruit flies representing 6 species collected from district Kulgam, with the population index being 0.18 fruit flies trapped/ day (Fig. 1). The adults observed were found to be characterized by the following: Head fulvous face with a pair of elongated facial spots less joined together towards the middle region, forming a transverse band. Thorax: Scutum is black and the scutellum is yellow in colour, with narrow black basal band. Postpronotol lobes and notopleura are yellow in colour. Mesopleural stripe is reaching almost to anterior notopleural seta dorsally. Wings have a narrow pale fuscous costal band confluent with $R_{2+3.}$ A small little bit elongated fuscous spot across apex of R_{4+5}. Wing cells bc and c are colourless, without microtrichia. Segmented legs with yellowish-brown colour, except hind tibiae that is brownish-gray apically. Abdomen: The abdomen is reddish behind the middle, with the presence of a black, narrow transverse and longitudinal band. Abdominal terga III-V is red brown with a black T-shaped pattern. Pecten on lateral side between III and IV terga is well developed. *Bactrocera. correcta* differs from *B. zonata* in possessing black scutum, and from *B. dorsalis* in possessing an incomplete costal band and transverse facial spots.

Bactrocera cucurbitae, *B. dorsalis*, *B. tau*, and *B. scutellaris* in Jammu and Kashmir had been reported during 2008-2009 from two districts, Budgam and Srinagar (Ganie et al., 2013); and *B. zonata*, had been added (Mir et al., 2014). Bhagat et al. (2014) reported *B. cucurbitae*, *B. zonata*, *B. tau*, and *B. dorsalis* from Kashmir. *Vidalia accola* Hardy (Mir and Mir, 2015) and *Hemilea malaisei* Hering (Mir et al., 2017) were added to these. Maximum adults of various *Bactrocera* spp. were observed during June to September on peach as reported by Jalaluddin (1996). In India, *B. correcta* is commonly found on guava fruits and reduces the total phenolic content of susceptible cultivars, and can cause 80% loss (Jalaluddin and Sadakathulla, 1999). *Bactrocera correcta*, often occurs along with *B. zonata* and these corroborate with the results of Kapoor (1989). *Bactrocera. correcta* is an exotic species, commonly found in India, Pakistan, Nepal, Vietnam, Myanmar, Thailand, China, Bhutan, and Sri Lanka (Drew, 2010). In many countries, it has been targeted as a quarantine pest as it is considered one of the highly invasive pests (Qin et al., 2016).

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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DEFENSE RESPONSES IN COWPEA ELICITED BY ENTOMOPATHOGENIC FUNGI AGAINST *APHIS CRACCIVORA* KOCH

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ABSTRACT

Use of entomopathogenic fungi for controlling the aphids is well attempted and further substantiation of interaction of entomopathogenic fungus in cowpea plants against aphid were studied in detail. Bioassays were performed with five fungal isolates against cowpea aphid *Aphis craccivora* Koch. The results revealed that the isolate *Beauveria bassiana* (NBAIR) recorded the highest mortality of 94.67% at 120 hr (at 5 days after treatment- DAT). The foliar application of its crude formulation @ 10^8 spores ml^{-1} had significant influence on the induction of defense related enzymes and components against *A. craccivora* infestation in cowpea. Resistant mechanism mediated by entomopathogenic fungi in cowpea against *A. craccivora* showed sustained and timely induction/ accumulation of defense enzymes. On 4 DAT the enzymes viz., peroxidases, polyphenol oxidases, phenylalanine ammonia lyase, chitinase, catalase and phenolics revealed maximum activity.

Key words: Cowpea, *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*, *Aphis craccivora*, defense, peroxidases, polyphenol oxidases, phenyl alanine ammonia lyase, chitinase, catalase, phenolics

Cowpea is the fourth most widely produced pulse crop in India after chickpea, pigeon pea and black gram. It can be grown during both rainy and summer seasons. The crop fits well into traditional rice-wheat cropping systems as a short duration crop and offers farmers with additional revenue. As a legume plant, it can play a significant role in fixing nitrogen from 20-80 kg/ ha (Hayat et al., 2008). Leguminous crops generally attract insect pests because of their high nutritive value (Mogotsi, 2006); therefore, proper and effective pest management methods should be adopted to minimize the losses caused by them (Singh et al., 2009). Aphid *Aphis craccivora* (Koch) is a major threat to cowpea from seedling to pod bearing stage. The nymphs and adults cause serious damage by sucking the plant sap. Young seedlings die as a result of high infestation, whilst older plants display signs such as stunted growth, crinkling, curling, late blooming and pods shriveling accompanied with yield loss. Aphid feeding damage to cowpea cultivars can result in production losses of up to 90% in susceptible varieties and up to 30% in intermediate resistance types. In modern agriculture, more selective and less polluting products are increasingly being employed by farmers for the effective control of insect pests (Siegwart et al., 2015).

Entomopathogenic fungi are the most abundant type of microorganisms that infects insect bodies through contact rather than ingestion and are widely distributed in nature across a wide host range and has immense significance because of environmental and food safety concerns. Furthermore, they are known to be non-toxic to non-target organisms like predators and parasitoids, and are easily cultured (Wu et al., 2015). They are especially significant in aphid management because aphids have morphological, biological, and ecological traits that render them vulnerable to fungal infections, which can produce epizootics that substantially lower aphid populations (Steinkraus, 2006). Among these fungi, *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Lecanicillium lecanii* (Zimmermann) Zare and Gams received major attention (Kalsbeek et al., 1995, Diaz et al., 2009). Increased interests in the use of entomopathogenic fungi in pest management necessitate the selection of fungal isolates with high virulence that show significant enzyme activities on target insects (Gebremariam et al., 2022). Keeping this in view, the current study evaluated entomopathogenic fungi for the control of *A. craccivora,* as well as the interaction between the effective entomopathogenic fungus in cowpea plants and *A. craccivora.*

MATERIALS AND METHODS

The study was carried out at the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore (11° 07' - 3.36" N, 76° 59' -

39.91" E). Cowpea cultivar 'Vamban 2 (VBN 2)' was used in all experiments for evaluating the efficacy of entomopathogens against *A. craccivora*. The entomopathogenic pure cultures strains of *Beauveria bassiana* (Bb NBAIR and Bb SBI), *Metarhizium anisopliae* (Ma NBAIR, and Ma SBI) and *Lecanicillium lecanii* (Ll NBAIR) were collected from the Department of Agricultural Entomology and Plant Pathology, Tamil Nadu Agricultural University (TNAU), National Bureau of Agricultural Insect Resources (NBAIR), Bangalore and Sugarcane Breeding Institute (SBI), Coimbatore. Sabouraud maltose agar with yeast extract (SMA+Y) medium (maltose 40 g, yeast extract 5 g, peptone 10 g, agar 15 g, distilled water 1l) were used to maintain the obtained colonies. To avert bacterial contamination, the antibiotics 0.05 mg ampicillin, 0.02 mg chloramphenicol and 0.2 mg streptomycin were added/ ml to the SMA+Y. The pure slants in test tubes were maintained at - 20°C as a stock culture. The apterous aphids' culture was established in the laboratory by collecting adults from the cowpea fields. To initiate the assay, cowpea plants were raised in mud pots containing potting mixture with sand, soil and dried cow dung in the ratio of 1:1:1. The aphids collected from the fields were relocated on the trifoliate stage of cowpea seedlings of VBN 2 variety. The pots were covered with netted cage and thereby the aphid colonies were kept free from natural enemies. Once in three weeks, new seedlings were raised and infested aphid twigs from the old plants were tied with them. Thus, aphid culture was maintained throughout the period of study (Saranya, 2012).

To test the pathogenicity of entomopathogens against aphid under laboratory conditions, fifteen days old VBN 2 seedlings were raised in paper cups measuring 7.5 x 7.5 cm. Six different spore concentrations ($1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ spores ml^{-1}) for each pure culture of entomopathogenic fungi (B. *bassiana, M. anisopliae, and L. lecanii*) were maintained individually. Each concentration was replicated thrice. For bioassay experiment, the apterous aphids were released on the cowpea seedlings @ 10 aphids/ seedling using camel hairbrush. Each treatment used a total of 30 aphids. After the aphids had been inoculated, an atomizer was used to spray the various concentrations ($1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ spores ml^{-1}) of fungal spore solutions on the seedlings. As a control, aphids were sprayed with a 0.05% Tween 80 solution (Butt and Goettel, 2000). Aphid mortality was monitored at 24 hr intervals for up to seven days. On daily basis, the dead aphids were collected and placed in a petridish with wet filter paper, which was then stored in a humid chamber. The mortality count included the deceased aphids that produced mycelial growth. Using Abbott's method, mortality data was adjusted for control mortality (Abbott, 1925).

Further substantiation of interaction of entomopathogenic fungus in cowpea plants were studied and the activity of defense enzymes i.e., biochemical alteration in plants were recorded at different time intervals. Cowpea leaves pretreated with a crude preparation of entomopathogenic fungal strains and challenged with aphids were collected for the estimation of defense related enzymes. Each replication of the treatment had four plants sampled separately. For comparison, untreated control (without spray, but infected with aphids) and absolute control (no aphid infestation and healthy) were also maintained. The leaves were collected at 24 hr interval initiating from 0 hr to 9 days. The obtained leaves were blended in liquid nitrogen and stored at -80°C. The homogenized sample was extracted with 2 ml of sodium phosphate buffer 0.1 M (pH 7.0) at 4°C. The obtained sample was centrifuged for 20 min at 10,000 rpm and the extract was used for estimation of defense enzymes. The chitinase (EC 3.2.1.14) colorimetric assay was performed according to the Boller and Mauch (1988). The enzyme activity was expressed as n moles GlcNAc equivalents min^{-1} g^{-1} fresh weight. The CAT activity (EC 1.11.1.6) was measured spectrophotometrically as reported by Chaparro-Giraldo et al. (2000). Activity was determined using the extinction coefficient (ε_{240nm} = 40 mM^{-1} cm^{-1}) for H2O2 and expressed in mmol min^{-1} g^{-1} of sample. The assay of phenol was calculated according to a standard curve obtained from a folin-ciocalteau reagent with a phenol solution (C6H6O) and represented as catechol equivalents g^{-1} tissue weight (Zieslin and Ben-Zaken, 1993).

RESULTS AND DISCUSSION

Pathogenicity of fungal isolates: Aphid mortality in treatments were corrected with respect to control mortality. Table 1 shows the data for corrected % mortality at various time periods. The highest mortality of 94.67% was obtained in the highest spore concentration of 10^8 spores ml^{-1} in *B. bassiana* (NBAIR) followed by *B. bassiana* (SBI) (92.44%) and *M. anisopliae* (NBAIR) (85.89%) at 120 hr (5 days) after treatment. At 144 hr (6 days) after treatment there were marked increases in the mortality. Among the fungal isolates, *B. bassiana* (NBAIR) and *B. bassiana* (SBI) recorded 100% mortality at 10^8 spores

Table 1. Effect of entomopathogenic fungal isolates on the mortality of *A. craccivora*

Entomopathogenic fungal Isolates	Spores ml^{-1}	% corrected mortality in hr					
		24	48	72	96	120	144
B. bassiana (Bb NBAIR)	10^8	7.89	34.22	58.67	88.78	94.67	100.00
	10^7	5.44	8.78	35.22	57.56	85.78	100.00
	10^6	0.00	6.56	23.56	52.89	78.56	80.00
	10^5	0.00	2.11	15.78	28.78	37.67	39.67
	10^4	0.00	0.00	14.33	18.67	22.00	28.89
B. bassiana (Bb SBI)	10^8	4.33	12.00	46.56	65.00	92.44	100.00
	10^7	0.00	5.56	24.44	43.33	82.22	85.56
	10^6	0.00	2.22	20.00	32.22	66.67	70.00
	10^5	0.00	0.00	12.22	21.11	30.00	33.33
	10^4	0.00	0.00	6.67	10.00	23.33	25.56
M. anisopliae (NBAIR)	10^8	0.00	15.33	28.78	54.11	85.89	94.22
	10^7	0.00	9.87	23.00	32.33	79.22	83.22
	10^6	0.00	4.33	12.11	28.56	54.78	64.33
	10^5	0.00	0.00	8.78	16.33	17.67	27.56
	10^4	0.00	0.00	4.56	8.67	14.33	18.78
M. anisopliae (SBI)	10^8	0.00	10.33	17.78	38.11	58.87	72.25
	10^7	0.00	7.48	13.00	28.33	32.22	65.22
	10^6	0.00	4.73	7.11	15.56	23.78	43.33
	10^5	0.00	0.00	5.78	12.33	16.67	25.56
	10^4	0.00	0.00	3.56	5.67	13.33	17.78
L. lecanii (Ll NBAIR)	10^8	0.00	11.33	25.78	41.11	58.89	62.22
	10^7	0.00	7.89	23.00	33.33	42.22	55.22
	10^6	0.00	0.00	11.11	25.56	37.78	43.33
	10^5	0.00	0.00	0.00	7.78	13.33	16.67
	10^4	0.00	0.00	0.00	6.67	12.33	15.78

ml^{-1}. With increased spore concentration, the duration of reproduction was significantly reduced. Within 24 hr of infection, the fertility of pea aphids, *A. pisum,* infected with *B. bassiana* and *P. neoaphidis* decreased (Baverstock et al., 2006). Smitha (2007) observed increase in mortality of banana mealy bug *Geococcus* sp (90 %) with *Hirsutella thompsonii* (10^8 spores ml^{-1}) after three days of inoculation. Ekesi et al. (2000) observed 91 and 93% mortality of *A. craccivora* at 7 days post treatment. Loureiro and Moino (2006) obtained 100% mortality of *Myzus persicae* by *B. bassiana* and *M. anisopliae* @10^6 and 10^7 spores ml^{-1}, respectively.

Plant defense induced by *B. bassiana* (NBAIR) against *A. craccivora*: The activitiy of the enzyme's phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, catalase and chitinase increased in the entomopathogenic fungal pathogens treated cowpea against *A. craccivora*, as demonstrated by biochemical analysis.

Peroxidase (PO): In the present study increased activity of PO was documented in *B. bassiana* (NBAIR) treated cowpea plants infested with *A. craccivora* and it was significantly different from all other treatments after 120 hr. The PO activity increased up to 120 hr of inoculation and thereafter, declined. No significant increase was noted in the untreated healthy plants (Table 2). PO's direct post-ingestive toxicity against insects has been related to production of semiquinone free radicals and ensuing synthesis of quinones (Zhu Salzman et al., 2008; Barbehenn et al., 2010). Peroxidases have been involved in different physiological functions that boosts plant resistance against herbivore and pathogen attack (Duffey and Stout, 1996); the process includes oxidation of hydroxyl cinnamyl alcohol, oxidation of phenol, cross linking of polysaccharide and extension monomers, lignification, production and polymerization of phenolics, hypersensitive response and suberization (Zhang et al., 2008; Chen et al., 2009). These phenomena in turn lead to the production of anti-nutritive compounds against the biotic stress (Gulsen et al., 2010; He et al., 2011).

Polyphenol oxidase (PPO): Accumulation of polyphenol oxidase was higher in *B. bassiana* (NBAIR)

Table 2. Induction of peroxidase (PO) and poly/ phenol oxidase (PPO) activity against aphid in *B. bassiana* treated cowpea

S. No.	Treatments	PO activity (changes in A420/ min/ g of fresh tissue) Hours after challenge inoculation						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	1.37^{a}	1.57^{a}	1.63^{a}	1.75^{a}	1.97^{a}	2.47^{a}	2.32^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	1.24^{ab}	1.45^{ab}	1.57^{ab}	1.74^{ab}	1.83^{b}	2.35^{ab}	2.28^{a}
3.	Chemical (dimethoate, check) (In)	0.92^{b}	1.32^{ab}	1.36^{b}	1.56^{b}	1.62^{b}	2.16^{b}	1.87^{bc}
4.	Control (Inoculated)	0.52^{cd}	0.57^{c}	0.62^{cd}	0.68^{cd}	0.68^{d}	0.68^{cd}	0.69^{de}
5.	Bb NBAIR (UI)	0.78^{bc}	0.72^{b}	0.82^{c}	0.93^{c}	1.24^{bc}	1.32^{c}	1.28^{bc}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	0.73^{bc}	0.78^{b}	0.85^{c}	0.96^{bc}	0.82^{cd}	1.23^{e}	0.87^{cd}
7.	Chemical (dimethoate) (UI)	0.65^{bc}	0.83^{b}	0.97^{bc}	0.94^{bc}	1.08^{c}	1.54^{bc}	1.47^{b}
8.	Healthy control	0.32^{d}	0.37^{cd}	0.38^{e}	0.45^{d}	0.52^{de}	0.57^{cd}	0.42^{e}
S. No.	Treatments	PPO activity (changes in A490/ min/ g of fresh tissue) Hours after challenge inoculation						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	0.23^{d}	1.27^{a}	1.37^{a}	1.58^{a}	1.67^{a}	1.92^{a}	1.82^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	0.85^{a}	1.12^{b}	1.18^{b}	1.48^{a}	1.59^{a}	1.73^{b}	1.68^{ab}
3.	Chemical (dimethoate, check) (In)	0.63^{b}	0.81^{bc}	0.84^{cd}	0.96^{b}	1.18^{b}	1.23^{bc}	0.97^{bc}
4.	Control (Inoculated)	0.52^{bc}	0.55^{c}	0.55^{d}	0.69^{bc}	0.76^{cd}	0.87^{d}	0.78^{cd}
5.	Bb NBAIR (UI)	0.44^{c}	0.53^{cd}	0.58^{d}	0.64^{c}	0.92^{c}	0.92^{cd}	0.94^{bc}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	0.45^{c}	0.52^{cd}	0.54^{d}	0.62^{c}	0.96^{bc}	0.95^{bc}	0.92^{c}
7.	Chemical (dimethoate) (UI)	0.38^{cd}	0.45^{d}	0.44^{e}	0.45^{d}	0.43^{e}	0.42^{e}	0.38^{e}
8.	Healthy control	0.32^{cd}	0.34^{de}	0.48^{e}	0.47^{d}	0.67^{de}	0.78^{de}	0.75^{cd}

In a column means followed by a common letter (s) not significantly different (p = 0.05, DMRT); In- Inoculated, UI- Uninoculated

treated plants challenge inoculated with *A. craccivora* and supreme activity was observed at 120 hr and thereafter started to decline whereas in uninoculated control plants, there was a slight induction of PPO (Table 2). PPO is an antinutritional enzyme that helps plants defend themselves against insect herbivory (Mahanil et al., 2008; Bhonwong et al., 2009). The process involves the formation of the highly reactive and toxic quinones from the phenols that interact with the amino acids chain nucleophilic side and causes cross-linking and restricts the protein supply to insect pests (Zhang et al., 2008; Bhonwong et al., 2009). PPOs create melanin, which improves the digestibility and palatability of plant tissues while also increasing cell wall confrontation to insects and pathogens (Zhao et al., 2009).

Phenylalanine ammonia lyase (PAL): In current study, entomopathogenic fungal bioformulation treated cowpea plants inoculated with *A. craccivora* induced the plant to synthesize higher level of PAL. The enzyme activity induced by *B. bassiana* (NBAIR) bioformulation reached the maximum at 120 hr and thereafter, it decreased (Table 3). In phenyl propanoid metabolism, phenyl alanine ammonia lyase (PAL) plays a crucial part in the production of several defensive compounds (Daayf et al., 1997). Plant-pathogen and plant-pest interactions may trigger PAL activity (Ramanathan et al., 2000; Radja Commare, 2000; Kandan et al., 2002; Bharathi et al., 2004; Harish, 2005; Saravanakumar, 2006). Corroborating to the above findings, when compared to an untreated healthy control, PAL activity was considerably greater in entomopathogenic fungal pathogen treatments supported by Harish (2005) and Saravanakumar (2006).

Chitinase (PR3): Chitinase activity was greater in the leaves of cowpea plants that had been pretreated with the *B. bassiana* (NBAIR) bioformulation and the enzyme activity peaked at 120 hours after infection (Table 3). Plants with high chitinase levels have been linked to pest and disease resistance (Maurhofer et al., 1994; Van Loon, 1997). It can also serve as an amylase inhibitor, preventing plant components from

Table 3. Induction of Phenylalanine Ammonia Lyase (PAL) and Chitinase activity against aphid in *B. bassiana* treated cowpea plants

S. No.	Treatments	PAL activity (n mol trans cinnamic acid/ min/ g of fresh tissue) Hours after challenge inoculation						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	7.44^{a}	7.87^{a}	8.25^{a}	11.32^{a}	14.28^{a}	18.02^{a}	18.32^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	7.33^{a}	7.38^{a}	8.09^{a}	10.09^{b}	12.14^{b}	15.19^{b}	15.24^{b}
3.	Chemical (dimethoate, check) (In)	6.22^{b}	6.27^{b}	6.62^{ab}	5.85^{c}	5.86^{cd}	7.15^{c}	8.26^{c}
4.	Control (Inoculated)	4.83^{c}	4.85^{cd}	5.17^{b}	3.24^{d}	4.63^{d}	3.28^{d}	3.22^{d}
5.	Bb NBAIR (UI)	1.38^{e}	1.58^{ef}	1.52^{cd}	0.53^{ef}	0.81^{e}	0.88^{f}	0.78^{g}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	1.35^{e}	1.53^{f}	1.48^{de}	0.50^{ef}	0.49^{ef}	0.99^{ef}	0.92^{ef}
7.	Chemical (dimethoate) (UI)	1.74^{d}	1.89^{e}	1.82^{c}	0.84^{e}	0.25^{f}	1.38^{e}	1.34^{e}
8.	Healthy control	1.57^{de}	1.56^{ef}	1.49^{d}	0.51^{ef}	0.46^{ef}	0.63^{fg}	0.58^{gh}
S. No.	Treatments	n mol GlcNac min^{-1} mg^{-1} of protein (Hours after inoculation)						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	6.42^{a}	5.42^{a}	5.76^{a}	6.74^{a}	6.34^{a}	6.34^{a}	6.12^{a}
2.	*Beauveria* talc based (commercial formulation Racer , check) (In)	5.15^{b}	5.37^{ab}	4.44^{b}	5.45^{b}	4.73^{b}	5.23^{b}	4.87^{b}
3.	Chemical (dimethoate, check) (In)	4.27^{bc}	4.28^{bc}	3.34^{c}	4.67^{bc}	3.92^{c}	3.86^{c}	3.82^{c}
4.	Control (Inoculated)	3.34^{cd}	3.22^{c}	2.36^{d}	3.45^{de}	2.65^{d}	2.75^{d}	2.74^{d}
5.	Bb NBAIR (UI)	3.12^{d}	3.20^{cd}	2.27^{d}	3.38^{d}	2.54^{de}	2.67^{de}	2.62^{d}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	2.29^{ef}	2.61^{d}	1.83^{e}	2.52^{f}	1.84^{e}	2.24^{e}	2.24^{e}
7.	Chemical (dimethoate) (UI)	2.26^{f}	2.39^{de}	1.56^{ef}	2.79^{ef}	1.73^{f}	1.84^{f}	1.82^{f}
8.	Healthy control	2.32^{e}	2.23^{e}	1.26^{f}	2.26^{g}	1.27^{g}	1.23^{g}	1.24^{fg}

In a column means followed by a common letter (s) not significantly different (p = 0.05, DMRT); In- Inoculated, UI- Uninoculated

being digested (Ary et al., 1989). Inducing chitinase activity might hinder the pest growth, alimentation and eventually kill it (Shapira et al., 1989; Wang et al., 1996).

Catalase (CAT): In response to *A. craccivora* inoculation, induction of catalase was higher in *B. bassiana* (NBAIR) bioformulation treated plants. The catalase activity was comparatively lower in the untreated plants challenged with *A. craccivora.* The extreme activity was noticed at 120 hr of infestation in *B. bassiana* (NBAIR) pretreated plants. In healthy plants, the expression was low (Table 4).

Phenolics: Increased phenolic accumulation was observed after *A. craccivora* was inoculated into *B. bassiana* (NBAIR) treated plants. On comparison, in the untreated healthy controls, phenol activity was rather modest. At 120 hr after infection, the highest accumulation was seen (Table 4).

Plants have evolved various defensive systems to defend themselves against herbivores and diseases. Insects are the most common herbivores, causing significant damage to plants, and protecting plants from these herbivores is critical for plant survival. Furthermore, the entomopathogenic fungal infections and their interaction readily influenced the sluggish growth of these insect pests and induce resistance. Feeny's slow-growth, high-mortality theory is supported by this observation (Feeny, 1976). Hence, it is concluded that slow development and high mortality is required, and certain entomopathogens should be considered as key natural enemies of insect pests.

CONFLICT OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

SP executed the research, B.M: Draft the paper, whereas PS supervised the work. All authors have read and agreed to the published version of the manuscript.

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Table 4. Induction of catalase and total phenolics activity against aphid in *B. bassiana* treated cowpea plants

S. No.	Treatments	Catalase activity (µmol of H_2O_2 consumed/ min/ g of fresh tissue) Hours after challenge inoculation						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	2.86^{a}	2.84^{a}	3.23^{a}	3.45^{a}	3.97^{a}	3.53^{a}	3.24^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	2.74^{ab}	2.76^{b}	3.14^{b}	3.21^{b}	3.33^{bc}	3.25^{b}	2.96^{b}
3.	Chemical (dimethoate, check) (In)	2.35^{b}	2.38^{b}	2.95^{c}	2.97^{c}	2.98^{c}	2.25^{c}	1.92^{c}
4.	Control (Inoculated)	2.26^{bc}	2.25^{c}	2.36^{d}	2.56^{d}	2.75^{d}	1.36^{d}	1.34^{cd}
5.	Bb NBAIR (UI)	1.44^{d}	1.56^{d}	1.58^{e}	1.67^{e}	1.85^{de}	1.12^{de}	0.92^{d}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	1.47^{d}	1.48^{de}	1.64^{e}	1.75^{e}	1.87^{ef}	0.94^{e}	0.85^{de}
7.	Chemical (dimethoate) (UI)	1.43^{d}	1.48^{de}	1.49^{f}	1.54^{e}	1.67^{f}	0.86^{f}	0.78^{e}
8.	Healthy control	1.48^{d}	1.45^{e}	1.47^{f}	1.48^{e}	1.54^{f}	0.68^{f}	0.54^{e}
S. No.	Treatments	Phenol content (µg of catechol/ g of fresh tissue) Hours after challenge inoculation *						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	92.18^{a}	104.45^{a}	108.37^{a}	118.95^{a}	124.47^{a}	128.44^{a}	121.43^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	86.72^{b}	97.44^{a}	98.24^{ab}	114.44^{a}	112.37^{b}	117.75^{b}	113.67^{b}
3.	Chemical (dimethoate, check) (In)	66.58^{d}	76.35^{b}	83.42^{c}	94.21^{bc}	92.22^{c}	97.86^{cd}	92.14^{cd}
4.	Control (Inoculated)	48.13^{e}	47.37^{c}	52.84^{d}	62.46^{d}	57.64^{e}	62.72^{e}	51.28^{e}
5.	Bb NBAIR (UI)	86.73^{b}	96.34^{a}	98.73^{ab}	108.15^{b}	103.75^{bc}	98.82^{cd}	99.85^{c}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	75.21^{c}	84.77^{c}	86.75^{bc}	92.98^{c}	87.23^{d}	82.75^{d}	87.87^{d}
7.	Chemical (dimethoate) (UI)	67.19^{d}	75.45^{b}	87.52^{bc}	98.84^{bc}	98.66^{c}	108.26^{c}	98.82^{c}
8.	Healthy control	40.31^{e}	42.36^{d}	45.83^{e}	52.82^{e}	43.83^{f}	41.65^{f}	38.24^{f}

In a column means followed by a common letter (s) not significantly different (p = 0.05, DMRT); In- Inoculated, UI- Uninoculated

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NEW HOST ASSOCIATIONS AND DISTRIBUTION RECORDS OF NATURAL ENEMIES OF BANANA PESTS

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ABSTRACT

New distribution records and host associations of natural enemies of banana insect and mite pests are reported. These include eight parasitoids (two trichogrammatids, two mymarids, two encyrtids, one pteromalid, one scelionid) and twelve predators (one thrips, two staphylinids and nine coccinellids). Two egg parasitoids, *Prosoligosita perplexa* Hayat & Husain and *Epoligosita duliniae* Livingstone and Yacoob are reported for the first time from banana tingid, *Stephanitis typica* (Distant). *Prosoligosita perplexa* is also recorded for the first time from South India and this is the first host record for this genus. Details of host associations are provided for the other bioagents recorded.

Key words: Banana, insect pests, mites, natural enemies, parasitoids, predators, scales, tingid, new record, distribution, India, Tamil Nadu.

Horticultural ecosystems provide ideal habitats for biological control in view of their perennial nature and the long duration of the crops. Biological control, naturally occurring or augmentative, is highly successful in perennial crops because of the undisturbed ecosystems that encourage the establishment and sustenance of natural enemies. Banana is a long duration fruit and vegetable crop and provides ideal conditions for the survival of a plethora of insect parasitoids, predators and also spiders. Among the pests of banana, only two weevil borers, *Cosmopolites sordidus* (Germar) and *Odoiporus longicollis* (Olivier), banana aphid, *Pentalonia nigronervosa* Coquerel, and the fruit scarring beetle, *Basilepta subcostata* (Jacoby), are considered as major pests in India, of which the last is confined to the northeastern and northern states. Unfortunately, very little work has been done in India in documenting the natural enemies of various banana pests and hardly any information is available in the literature. This paper documents the results of long-term observations on the parasitoids and predators of banana pests mainly from Tamil Nadu and some other parts of India.

MATERIALS AND METHODS

The specimens were collected during the surveys conducted for insect and mite pests of banana and their natural enemies during 2016–20 in Tamil Nadu and Uttar Pradesh. Field collected insect and mite pest stages were brought to the laboratory and the natural enemies reared from various life stages were collected, preserved and identified by using the available keys. Photographs of the natural enemies were taken with a Nikon D750 DSLR camera and Leica DMC 4500 digital camera attached to a Leica M205A stereozoom microscope. The specimens are preserved in the reference collections maintained at the National Research Centre for Banana, Trichy.

RESULTS AND DISCUSSION

In the surveys, totally eight parasitoids (two trichogrammatids, two mymarids, two encyrtids, one pteromalid, one scelionid) and twelve predators (one thrips, two staphylinids and nine coccinellids) were identified in association with various insect and mite pests. The host associations of these natural enemies are given below.

A. Parasitoids

Hymenoptera

a. Trichogrammatidae

***Prosoligosita perplexa* Hayat & Husain** (Fig. 1a, c): *Prosoligosita perplexa* was recorded as an egg parasitoid of the banana tingid, *Stephanitis typica* (Distant) from Tamil Nadu. It was originally described based on female specimens and it is known to be distributed in Madhya Pradesh, Odisha, and Uttar Pradesh (Hayat and Husain, 1981; Noyes, 2019). Until now, its host associations were not known. The host

association with *S. typica* and the locality (Tamil Nadu) are new records for this genus and species.

Epoligosita duliniae **Livingstone and Yacoob (1986)**(Fig. 1b, d): It was recorded as an egg parasitoid of the banana tingid from Tamil Nadu. This parasitoid is known to be distributed in Kerala and Tamil Nadu and parasitizes the morinda tingid, *Dulinius conchatus* Distant (Livingstone and Yacoob, 1983; Noyes, 2019). Its association with *S. typica* constitutes a new host record. Both *Epoligosita duliniae* and *P. perplexa* were found to coexist on banana tingid colonies and difficult to differentiate in view of their similar coloration and very small size (Length: 0.5-0.6 mm). The forewing venation is a useful character to separate *E. duliniae* (Fig. 1c) and *P. perplexa* (Fig. 1d).

b. Mymaridae

Erythmelus **sp.** (Fig. 1e): Another egg parasitoid belonging to *Erythmelus* was collected in association with banana tingid from Tamil Nadu and it could not be matched with any of the known species. *Erythmelus panis* Enock, distributed in different parts of Europe, Africa and India, is known to be a parasitoid of the pear tingid, *Stephanitis pyri* (Fabricius) (Noyes, 2019).

Lymaenon **sp.** (Fig. 1f): *Lymaenon* sp. was collected from banana tingid colonies from Tamil Nadu and it is likely to be a parasitoid of tingid eggs.

c. Encyrtidae

Aenasius advena **Compere** (Fig. 1g): It is a commonly collected parasitoid of the tailed mealybug, *Ferrisia virgata,* a pest of banana foliage and fruits, in Tamil Nadu. It is new to the banana ecosystem as per the Universal Chalcidoidea Database (Noyes, 2019).

Blepyrus insularis **(Cameron)** (Fig. 1h): It is an endoparasitoid of *F. virgata,* a pest of banana foliage and fruits, in Tamil Nadu but never collected in large numbers. There is no previous record of its association with *F. virgata* infesting banana (Noyes, 2019).

d. Pteromalidae

Cephaleta brunniventris **Motschulsky** (Fig. 1i): This species is a commonly collected parasitoid of soft scales (Hemiptera: Coccidae) (Sureshan et al., 2011). It was reared from *Saissetia* sp. infesting banana in and around Trichy, Tamil Nadu. There is no record of its occurrence in banana ecosystem as per Noyes (2019).

e. Scelionidae

Telenomus **sp.** (Fig. 1j): *Telenomus* sp. was recorded as an egg parasitoid of *Amata passalis* (F.)*,* a minor pest of banana foliage in Tamil Nadu.

B. Predators

a. Thysanoptera: Thripidae

Scolothrips **sp.** (Fig. 2a): *Scolothrips* sp., close to *S. rhagebianus* Priesner, was recorded in association with all the major mite pests of banana, particularly *Oligonychus* spp., *Eutetranychus orientalis* (Klein) and *Raoiella indica* (Hirst)*,* almost throughout the year in Tamil Nadu. *Scolothrips* spp. are well known predators of phytophagous mites (Mound, 2011).

b. Coleoptera: Staphylinidae

Oligota **sp.** (Fig. 2b): Larvae of *Oligota* sp. were found to be commonly associated with tetranychid mites infesting banana, particularly *Eutetranychus orientalis,* in Tamil Nadu. The species could not be identified because the larvae were not reared to adults.

Paederus fuscipes **Curtis:** It is a major polyphagous predator of many agricultural pests in India and was found in association with banana fruit scarring beetle in and around Lucknow, Uttar Pradesh.

c. Coleoptera: Coccinellidae

Parastethorus indira **(Kapur)** (Fig. 2c): It was a specific predator of *Eutetranychus orientalis* and commonly found during summer in Tamil Nadu.

Stethorus **spp.:** Four species of *Stethorus* were recorded as predators of banana mites in many parts of Tamil Nadu and found to be highly host-specific. *Stethorus pauperculus* Weise (Fig. 2d), *S. forficatus* Poorani (on *Oligonychus* spp. and *Tetranychus* spp.), *S. keralicus* Kapur (on *Raoiella indica*) (Fig. 2e), and one undescribed species of *Stethorus* (on tetranychids) were commonly collected from the field germplasm bank of ICAR-NRCB, Trichy. Of these, *S. pauperculus* was found to be the most abundant predator in the banana ecosystem and collected round the year whereas the other species were found in comparatively fewer numbers and their activity coincided with the summer months (April-August) when mite population levels were at their peak.

Chilocorus nigrita **(F.)** (Fig. 2f): This is the most common and effective predator of banana scale, *Aspidiotus destructor* Signoret (Hemiptera: Diaspididae) in South India and found almost round the year except winter (November-February).

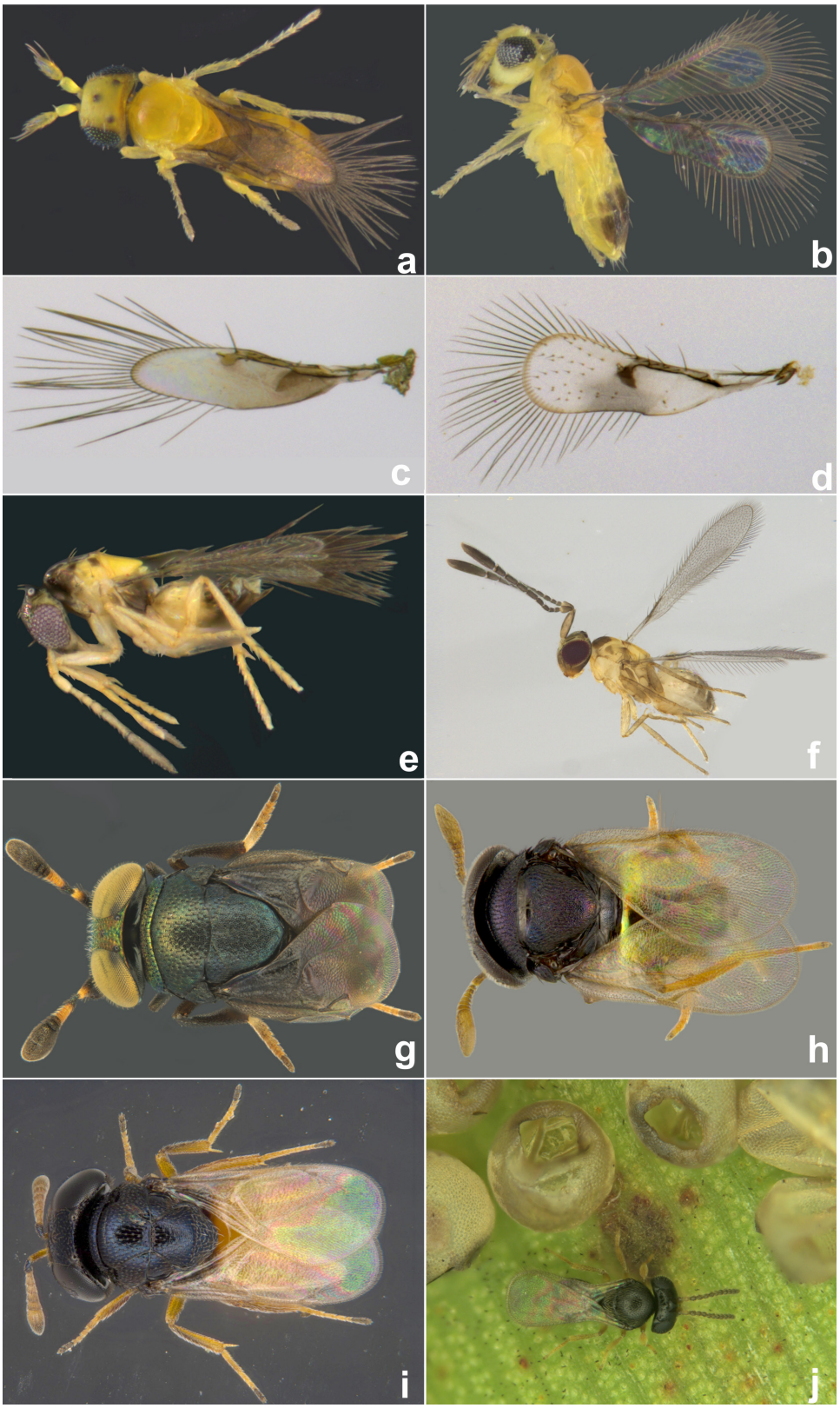


Fig. 1. Parasites of insect and mite pests of banana a. *Epoligosita duliniae*; b. *Prosoligosita perplexa*; c. Forewing, *Epoligosita duliniae*; d. Forewing, *Prosoligosita perplexa*; e. *Erythmelus* sp.; f. *Lymaenon* sp.; g. *Aenasius advena*; h. *Blepyrus insularis*; i. *Cephaleta brunniventris*; j. *Telenomus* sp.

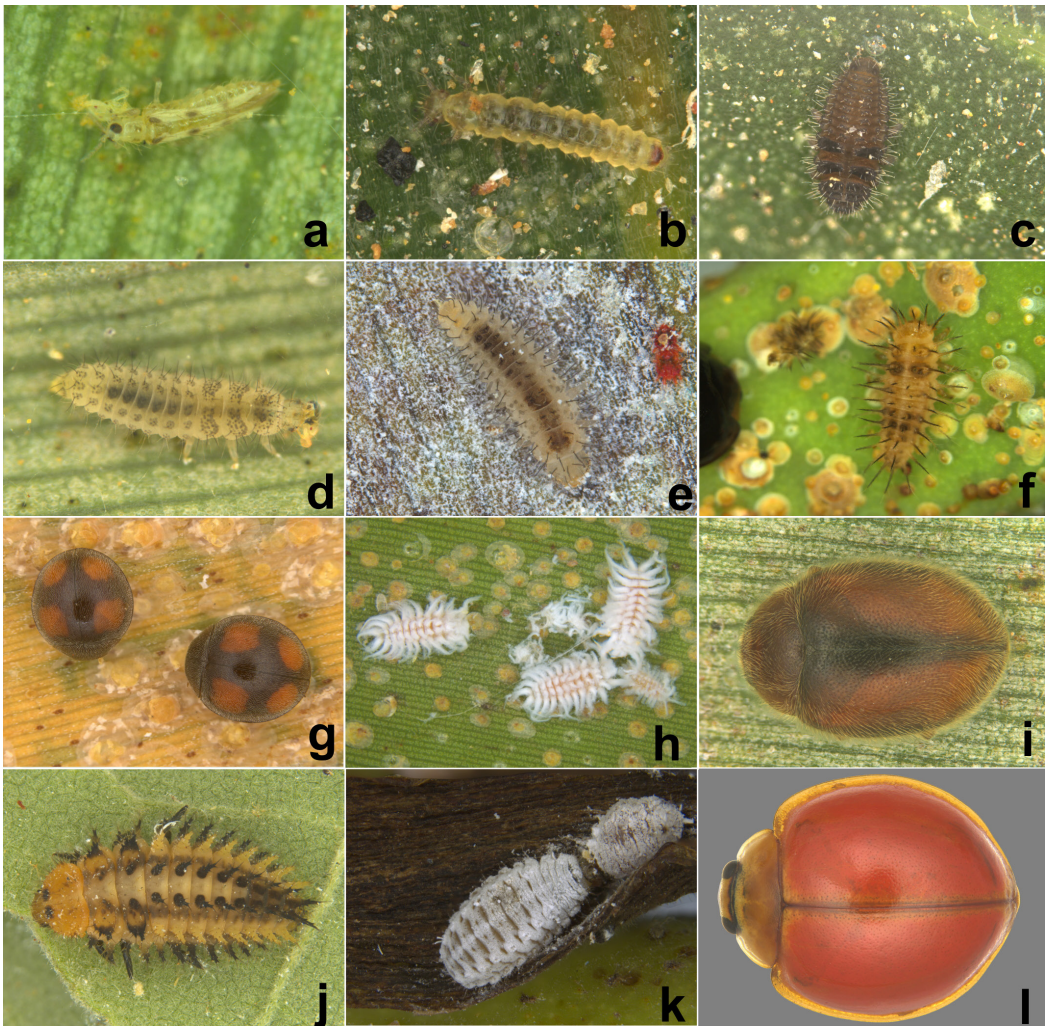


Fig. 2. Predators of insect and mite pests of banana: a. *Scolothrips* sp.; b. *Oligota* sp.; c. *Parastethorus indira*; d. *Stethorus pauperculus*; e. *Stethorus keralicus*; f. *Chilocorus nigrita*; g. *Pharoscymnus horni*; h. *Sasajiscymnus dwipakalpa*; i. *Scymnus fuscatus*; j. *Brumoides suturalis*; k. *Hyperaspis maindroni*; l. *Micraspis yasumatsui*

***Pharoscymnus horni* (Weise)** (Fig. 2g): Larvae and adults of *P. horni* were found to feed on *A. destructor* and other diaspine scales on banana during summer (April-May) in and around Trichy, Tamil Nadu, and were not commonly collected.

***Sasajiscymnus dwipakalpa* (Ghorpade)** (Fig. 2h): Larvae and adults of *S. dwipakalpa* were found to feed on *A. destructor* mainly during the summer (April-August) in Tamil Nadu. It is an effective and highly specific predator of *A. destructor* (Ghorpade, 1977) and widely distributed in South India.

Scymnus* (*Neopullus*) *fuscatus (Fig. 2i): It coexists with *S. nubilus* in the banana ecosystem in association with *A. destructor* and other scales in Tamil Nadu. It closely resembles *S. nubilus*, which is an effective predator of banana aphid (Poorani et al., 2022), and can be differentiated by its distinctly denser and longer dorsal pubescence.

***Brumoides suturalis* (F.)** (Fig. 2j): Larvae of *B. suturalis* were found to feed on *Phenacoccus solenopsis* Tinsley, an occasional pest of banana in and around Trichy, Tamil Nadu.

***Hyperaspis maindroni* Sicard** (Fig. 2k): Larvae and adults of *H. maindroni* were found to feed on *Dysmicoccus brevipes* (Cockerell) and solenopsis mealybug infesting banana bunches and foliage in Tamil Nadu.

***Micraspis yasumatsui* Sasaji** (Fig. 2l): Several specimens of *Micraspis yasumatsui* were collected from the leaf whorl and sheath of banana near rice fields in and around Lucknow, Uttar Pradesh, during October

2018. *Micraspis* spp. are abundant in rice ecosystem and it is most likely that banana is used as a shelter/ refugium by this species because the banana plants were free of pests.

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CONFLICT OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

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DIVERSITY OF ARTHROPODS IN RELATION TO INSECTICIDE AND BIOPESTICIDE TREATMENTS IN OKRA

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ABSTRACT

Present study was carried out during 2019 at the Vegetable Research Farm, Punjab Agricultural University, Ludhiana to generate information on diversity of invertebrates in okra. Study revealed that okra crop was harboured by 27 invertebrate species belonging to 11 orders and 26 families. Of these, maximum belonged to order Hemiptera followed by Coleoptera. Higher values of Shannon-Wiener index (1.75, 1.84) and Simpson's index (0.26, 0.17), respectively were recorded for both phytophagous arthropods and predators in untreated control plots. This is followed by neem/ other insecticides treated plots. Imidacloprid lowered the arthropod population. Occurrence of predators and phytophagous arthropods revealed a significant positive correlation with temperature (r=+0.37 to +0.99) and relative humidity (r=+0.04 to +0.80) except for few hemipterans and coleopterans.

Key words: Okra, arthropods, insects, diversity, biopesticide, insecticides, neem, phytophagous, predators, Diversity indices, population dynamics, weather factors

Biodiversity refers to the number and variety of living organisms that live in a particular area or across the world, and provides economic benefits and ecological stability (Iqbal et al., 2018). Okra (*Abelmoschus esculentus* L.) is an important vegetable crop infested by a large variety of arthropods. It plays a vital role in the human diet (Adeboye and Opunta, 1996; Baloch *et al.*, 1990). Low yield of okra is due to damage caused by many arthropods (Rachana et al., 2009) and species diversity of arthropods varies from region to region. About, 72 arthropods infest okra crop (Rao and Rajendran, 2002), however, those causing economic loss include whitefly, shoot and fruit borer and thrips (Solangi and Lohar, 2007). Some others like red cotton bug are of minor importance (Dhamdhere et al., 1984); green semilopper, cotton stem weevil and pink bollworm. Borer and sucking arthropods are the major constraints; 91.60% loss is caused by *Earias* spp. and *Helicoverpa armigera* (Hubn.) (Pareek and Bhargava, 2003); 48.97% damage was reported due to insect pests (Kanwar and Ameta, 2007); 74.00% loss due to damage by sucking arthropods is known, of which, about 40.00% was due to leafhoppers (Rawat and Sahu, 1973). Among sucking arthropods whitefly is the major problem as a vector of leaf curl virus and causing 54.00% reduction in yield (Rai et al., 2014); spider mite caused 17.46% reduction in yield and it is a major pest (Mandal et al., 2006). Besides these, cotton aphid, red cotton bud and blister beetle were also reported (Barwal and Rao, 1988).

In okra crop, population of phytophagous arthropods and non arthropods have been influenced by weather parameters such as temperature and relative humidity. The abundance and rate of built up of these can be understood by study of their interaction with weather factors (Khan et al., 2001). Injudicious use of pesticides had resulted in development of resistance to pesticides and also have adverse effects on predators, resurgence of minor arthropods, environmental pollution, hazards to economy and health (Mandal et al*.,* 2006). Although, the use of pesticides cannot be avoided, yet their use can be limited by including biopesticides. Different neem extracts have been reported as ecofriendly option in okra (Bindu et al., 2003). Neem formulations have no toxic effects and are least toxic to non target organisms and have very less chances of development of resistance as these act as growth inhibitor, oviposition deterrent and antifeedant (Patel et al., 1996). However, information is scanty regarding invertebrates/ arthropods present in okra. Hence, this study to have a more detailed and consolidated account of invertebrates/ arthropods present on okra crop field under both sprayed and unsprayed conditions.

MATERIALS AND METHODS

The present surveys were carried out at the Vegetable Farm, PAU, Ludhiana from July to December 2019 on okra (variety Punjab Suhawani). The field was levelled and was divided into plots of size (3 x 4 m) as per layout

in RBD design. Sowing was done on 2th July, 2019 with a recommended row to row 45 cm and plant to plant 30 cm distance. Recommended agronomic practices were followed. Insecticides evaluated include- flonicamid 50%WP @ 0.8 g/ l of water (ulala), imidacloprid 200SL @ 0.4 ml/ l of water (Confidor), spiromesifen 240SC @ 1.5 ml/ l of water (Oberon); and biopesticide neem bhan (50,000 ppm) @ 0.8 ml/ l of water (azadirachtin 5%EC). Treatments were allocated to plots on random basis, with sprays done duing 2nd week of August and 1st week of September, with knapsack sprayer. For counts of invertebrates visiting okra, five plants/ plot were selected randomly (belonging to four sides as well as from the centre of field) and from each plant three leaves were randomly selected each from upper, middle and lower leaves (nine leaves/ plant). The counts were made at weekly intervals from germination till harvest, with invertebrates searched visually and collected by using aspirator, sweep net, pitfall method and pheromone trap. Sweep net method was used to count the abundance of flying insects for which sweeping was done back and forth through the plant canopy five times in each plot (Newman, 1835). Specimens collected were sorted, counted, photographed and identified using suitable keys. The collected invertebrates were killed with ethyl acetate. Large invertebrates were pinned and preserved in insect collection box, whereas small invertebrates were preserved in 70% alcohol. Weather data was obtained from the meteorological observatory (Department of Climate Change and Agricultural Meteorology, Punjab Agricultural University, Ludhiana). Occurrence of invertebrates was correlated with temperature and relative humidity. To calculate biodiversity indices like relative abundance, Simpson index, Shannon- Wiener Index and species evenness standard formulae were followed.

The mean values of occurrence of arthropods and non arthropods were calculated and correlated with temperature and relative humidity using correlation regression analysis. Data was analysed using one way of ANOVA to record the efficacy of treatments along with control. Comparison was made between different treatments using Tukey's test.

RESULTS AND DISCUSSION

As regards, diversity of phytophagous invertebrates The results from the weekly surveys revealed the occurrence of 27 species belonging to 11 orders and 26 families (Table 1); Hemiptera with maximum species (eight) followed by Coleoptera (six). Hemipterans recorded were jassids, whitefly, coreid bug, mealy bug, dusky cotton bug, red cotton bug, sugarcane leaf hopper, stink bug among which whitefly and jassid were predominant. Lepidoptera found on okra were okra fruit and shoot borer, tobacco cutworm, and American bollworm, and Coleoptera include- red pumpkin beetle, blister beetle, ash weevil, white spotted leaf beetle and chafer beetle. Among predators, lady bird beetle, dragonfly, damselfly, rober fly, yellow wasp, spider, and praying mantid were found. Nair et al. (2017) in okra from Tripura, India revealed Hemiptera as the most abundant group followed by Coleoptera. Bhatt et al. (2018) also found Hemiptera as the most abundant at Pantnagar (Uttarakhand). Ruhul Amin et al. (2019) reported that Hemiptera was most abundant in Gazipur (Bangladesh).

Diversity indices revealed higher value of species richness (19.00) for phytophagous arthropods in untreated control plots; and flonicamid, imidacloprid and neem baan treated ones. The value of evenness recorded was 0.59, 0.58, 0.56, 0.55 and 0.54 for control, neem baan, spiromesifen, flonicamid and imidacloprid treated okra plots. This indicates a decrease in trend of evenness in phytophagous arthropods. The Shannon–Wiener index (H') for the phytophagous arthropods was 1.75, 1.60, 1.67, 1.59 and 1.71 for control, spiromesifen, flonicamid, imidacloprid and neem baan treated plots, respectively. The data indicates that control and neem baan treated okra fields diverse followed by spiromesifen treated fields whereas, fields treated with flonicamid and imidacloprd were less diverse as compared to control. The Simpson's index (D) for control, spiromesifen, flonicamid, imidacloprid and neem baan treated plots was recorded to be 0.26, 0.30, 0.28, 0.33 and 0.27, respectively indicating high degree of diversity in control and neem baan treated plots. The value of evenness for predators was 0.88, 0.85, 0.78, 0.75 and 0.75 for control, neem baan, spiromesifen, flonicamid and imidacloprid treated okra fields, respectively which indicate a decrease in trend of evenness; Shannon–Wiener index (H') was 1.85, 1.63, 1.53, 1.57 and 1.76 for control, spiromesifen, flonicamid, imidacloprid and neem baan treated fields, respectively. The data indicates that control and neem baan treated fields were more diverse followed by spiromesifen treated fields whereas, fields treated with flonicamid and imidacloprid were less diverse as compared to control. The Simpson's index (D) for control, spiromesifen, flonicamid, imidacloprid and neem baan fields was 0.17, 0.21, 0.23, 0.23 and 0.29, respectively indicating high degree of diversity (Table 1).

Table 1. Diversity, richness and evenness under sprayed and unsprayed conditions in okra

Order	Species	Occurrence				
		Control	Spiromesifen	Flonicamid	Imidacloprid	Neem baan
Phytophagous arthropods						
Coleoptera	Ash weevil	0.78	0.12	0.06	0.06	0.18
	Red pumpkin beetle	1.09	0.06	0.06	0.12	0.12
	Blister beetle	1.56	0.82	0.62	0.31	0.70
	Chafer beetle	3.90	0.70	0.38	0.24	1.77
	White spotted leaf beetle	0.96	0.12	0.18	0.06	0.18
Hemiptera	Stink bug	2.75	0.63	0.57	0.76	1.09
	Whitefly	94.07	4.67	3.58	3.70	8.50
	Jassid	131.87	37.88	20.21	30.81	46.42
	Red cotton bug	2.77	1.23	0.85	1.11	1.87
	Dusky cotton bug	2.84	1.18	0.91	0.97	1.44
	Mealy bug	2.12	0	0.46	1.46	0.66
	Sugarcane leafhopper	0.51	0.06	0.12	0.06	0.12
Lepidoptera	Rice stem borer	0.59	0.18	0.06	0.06	0.26
	Coreid bug	1.23	0.13	0.06	0.12	0.19
	Okra fruit and shoot borer	7.83	2.12	1.97	1.63	2.80
	American bollworm	13.23	4.36	2.58	4.03	5.63
Trombidiformes	Red spider mite	49.03	11.68	20.9	26.96	19.88
Stylommatophora	Snail	6.21	3.87	3.57	2.90	4.28
Orthoptera	Grasshopper	3.10	0.06	0.06	0.13	1.17
Species richness		19.00	18.00	19.00	19.00	19.00
Species evenness		0.59	0.56	0.55	0.54	0.58
Simpson's index		0.26	0.30	0.28	0.33	0.27
Shannon-Wiener index		1.75	1.60	1.67	1.59	1.71
Predators						
Coleoptera	Lady bird beetle	5.95	2.48	2.15	2.10	5.26
Araneae	Spider	4.62	2.76	2.62	1.95	4.08
Hymenoptera	Yellow wasp	1.09	0.38	0.18	0.12	0.75
Diptera	Robber fly	1.10	0.12	0.12	0.06	0.63
Odonata	Dragonfly	4.36	2.49	2.16	1.56	3.51
	Damselfly	4.22	2.69	2.23	1.63	3.62
Orthoptera	Silent leaf runner	2.10	0.19	0.06	0.06	1.10
Dictyoptera	Praying mantid	0.31	0.06	0.06	0.06	0.25
Species richness		8.00	8.00	8.00	8.00	8.00
Species evenness		0.88	0.78	0.75	0.75	0.85
Simpson's index		0.17	0.21	0.22	0.23	0.19
Shannon-Wiener index		1.84	1.63	1.57	1.57	1.76

The occurrence of phytophagous arthropods was observed to be in a non-significant positive or negative correlation with relative humidity as given in Table 2; Purohit et al. (2006) reported a positive correlation with jassids, while Ghuge et al. (2020) with evening relative humidity showed a non-significant positive correlation with whitefly at Parbhani (Maharashtra); Gupta et al. (1998) and Ghuge et al. (2020) observed a positive correlation of fruit and shoot borer with relative humidity. Mandal et al. (2006) reported such a positive correlation of red spider mite. Ghuge et al. (2020) reported that *H. armigera* was exhibiting a a non-significant positive correlation. As far as temperature is concerned, there was a non-significant negative correlation (r=-0.03 to -0.82) with many arthropods. Jat and Singh (2019) revealed that there was a negative correlation with maximum temperature for jassids in Rajasthan; Khating et al. (2016) reported a negative correlation with minimum temperature. With predators mean relative humidity showed a non-significant positive correlation (r= +0.06 to +0.74) as shown earlier (Singh et al., 2020; Nayer et al., 2017; Shukla et al., 2014; Dhaka and Pareek, 2007). For predators, it was a non-significant negative correlation with mean temperature for some, however, it was r=+0.88 to +0.99 for dragonfly, damselfly and silent leaf runner, and

Table 2. Correlation coefficients- phytophagous arthropods and predators in okra vs RH and Temperature

Species	Mean incidence/ occurrence				RH Correlation coefficient (r)	Temperature Correlation coefficient (r)
	July	Aug	Sept	Oct		
Blister beetle	0.01	0.23	0.13	0.01	+0.69	+0.57
Chafer beetle	0.03	0.47	0.46	0	+0.80	+0.60
White spotted leaf beetle	0	0	0.19	0.04	+0.26	-0.03
Stink bug	0	0.09	0.48	0.11	+0.37	+0.06
Whitefly	0.73	11.66	9.53	1.66	+0.71	+0.51
Jassid	0.43	9.21	15.53	7.79	+0.30	-0.03
Red cotton bug	0	0.13	0.38	0.18	+0.19	-0.13
Dusky cotton bug	0	0	0.34	0.21	-0.15	-0.41
Mealy bug	0	0	0.34	0.18	-0.03	-0.33
Coreid bug	0	0.09	0.19	0.01	+0.66	+0.37
Okra fruit and shoot borer	0	0.84	1.06	0.04	+0.74	+0.49
American bollworm	0.01	1.71	1.48	0.09	+0.74	+0.53
Red spider mite	1.76	6.73	3.06	0.69	+0.70	+0.67
Snail	0.03	0.83	0.63	0.06	+0.73	+0.59
Grasshopper	0	0.44	0.28	0.04	+0.65	+0.49
Red pumpkin beetle	0	0.03	0.80	0.06	+0.24	-0.80
Ash weevil	0	0	0.08	0.11	-0.59	-0.82
Sugarcane leafhopper	0	0.03	0.09	0	+0.65	+0.37
Rice stem borer	0	0	0	0.14	-0.96*	-0.98*

Predators vs relative humidity

Species	Mean occurrence				Correlation coefficient (r)
	July	Aug	Sept	Oct	
Lady bird beetle	0.08	0.58	0.64	0.18	+0.06
Spider	0.03	0.48	0.54	0.09	+0.72
Yellow wasp	0.03	0.14	0.06	0.03	+0.71
Robber fly	0	0	0.19	0.08	+0.04
Dragonfly	0.03	0.54	0.44	0.06	+0.74
Damselfly	0	0.41	0.53	0.11	+0.64
Silent leaf runner	0	0.21	0.26	0.04	+0.69
Praying mantid	0	0.03	0.03	0.01	+0.60

Predators vs temperature

Species	Mean occurrence				Correlation coefficient (r)
	July	Aug	Sept	Oct	
Lady bird beetle	0.08	0.58	0.64	0.18	+0.99**
Spider	0.03	0.48	0.54	0.09	+0.95*
Yellow wasp	0.03	0.14	0.06	0.03	+0.93*
Robber fly	0	0	0.19	0.08	-0.07
Dragonfly	0.03	0.54	0.44	0.06	+0.99*
Damselfly	0	0.41	0.53	0.11	+0.90*
Silent leaf runner	0	0.21	0.26	0.04	+0.92*
Praying mantid	0	0.03	0.03	0.01	+0.88

*Significant at p=0.05

significant. Similar results were obtained by Dwivedi et al. (2018), Nager et al. (2017), Mouly et al. (2018), Patel et al. (2005), Dhaka and Pareek (2007) and Bhatt et al. (2018).

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AUTHOR CONTRIBUTION STATEMENT

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CONFLICT OF INTEREST

No conflict of interest.

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OPTIMAL MECHANICAL EXCLUSION DEVICES FOR MANAGEMENT OF KHAPRA BEETLE IN STORED WHEAT

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ABSTRACT

The effect of mechanical exclusion devices and its number in combination viz. probe trap (A; 1-3), two-in-one trap with 3 mm perforations (B; 1-5) and two-in-one trap with 2 mm perforations (C; 1-3) were investigated to test the efficiency of these traps for trapping larvae and adults of khapra beetle *Trogoderma granarium* Everts. Colour change and weight loss (%) happening in wheat stored in bins of 50 kg capacity for six months were used as the criteria. The experiments were planned using a three-factor-three-level Box and Behnken design of response surface methodology and were optimized for maximum trapping, with minimum change in colour and weight loss. Analysis of variance (ANOVA) revealed significantly ($p \leq 0.05$) higher effects of quantity (number) of two in one trap with 3 mm perforations followed by two in one trap with 2 mm perforation on total trapping efficiency. Colour change and weight loss in stored wheat exhibited a negative relationship with trap capture. Among the process parameters studied, the probe trap and its number in experimental combination demonstrated a significantly lower effect on the response variables. The optimal combinations of mechanical exclusion process parameters obtained by numerical optimization technique for trapping larvae and adults were 3:4:3 and 2:5:3 (probe trap: two in one model trap 3 mm: two in one model trap 2 mm) with desirability of 0.61 and 0.63, respectively.

Key words: *Trogoderma granarium*, mechanical exclusion, combinations, probe trap, two-in-one traps, perforations, trap capture, numerical optimization, colour change, weight loss

The khapra beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is one of the most noxious and quarantine insect-pest of food grains and other stored food products (Athanassiou et al., 2019). It is considered one of the 100 worst invasive species in the world. Infestation caused by khapra beetle are difficult to control because these survive without food for long periods and also as these prefer dry conditions and have resistance to many insecticides (Ali et al., 2012). Therefore, it is necessary to evaluate alternate strategies against, one of them is use of mechanical exclusion devices which has been developed by the Tamil Nadu Agricultural University (TNAU) (Mohan et al., 2004). Mechanical exclusion devices viz. probe trap and two-in-one trap have significantly reduced the damage in wheat and pulses by trapping *Sitophilus granarius* (L.) and bruchids, respectively (Debebe et al., 2008). There are probe pit fall trap for adults of *Cryptolestes ferrugineus* (Stephens) in wheat filled containers (Mohan et al., 2008), TNAU stack probe trap for *Lasioderma serricorne* (F.) in turmeric, TNAU two-in-one trap for bruchids in pulses are well documented (Subramanyam and Harein, 1990; Debebe et al., 2008; Rajesh et al., 2015), probe trap and two-in-one trap for *T. granarium* in stored wheat and rice (Anonymous, 2017, 2018a). But the number of these traps needs to be optimised for their use in wheat stored in bulk in stored bins. Keeping in view, the present study was planned to optimize the type and number of mechanical exclusion devices in combination against management of *T. granarium* in stored wheat by using response surface methodology (RSM) which is an effective statistical technique for optimizing complex processes (Yagci and Gogus, 2008; Alam et al., 2011).

MATERIALS AND METHODS

In order to assess the effect of mechanical exclusion devices and to optimize its type and number against khapra beetle in stored wheat, present investigations were conducted during the year 2019-20 and 2020-21 in the Department of Processing and Food Engineering, Punjab Agricultural University (PAU), Ludhiana. For experimentation, the wheat grains were procured from the Research Farm of Department of Agronomy, PAU, Ludhiana. The mixed age populations of *T. granarium* were collected from different locations of Distt. Ludhiana, Punjab. The wheat grains used for rearing *T. granarium* were disinfested at 60ºC for one

hour so as to make them free from any other insect infestation (Mookherjee et al.,1968). Later, these wheat grains were kept in jars having 1 kg capacity and were artificially infested with the larvae of *T. granarium* to get the pure culture. These jars were placed in BOD incubators maintained at 29±2 ºC and 70±5% RH for the multiplication of *T. granarium*. For long term storage, wheat grains were disinfested as per recommended package of practices (Anonymous 2018b) and then filled in 15 bins of 50 kg capacity. These wheat grains were infested with 20 pairs of *T. granarium* which showed good movement, then left it for 30 days for its multiplication.

The mechanical exclusion devices viz. probe trap, two-in-one trap with 3mm and 2mm perforations selected for the study were procured from Tamil Nadu Agricultural University (TNAU) as given in Fig. 1 a-c. Response surface methodology (RSM) was adopted in the design of experimental combinations (Altan et al., 2008; Yagci and Gogus, 2008) with an advantage of reduced number of experimental runs needed to provide sufficient information for statistically acceptable results. The independent variables with three different levels viz. -1, 0 and +1 which formed a total of 15 combinations (Giles et al., 2004) which included three replicates of the centre point each sighed the coded value 0. Response variables were larvae and adult trapped, colour change and weight loss. The data on number of trapped larvae/adults of *T. granarium* were recorded by calculating the total mean trapping efficiency of larvae/adults (TMTE L/A) and the proportion of larvae/adults trapped in different traps. Thus, to calculate total mean trapping efficiency of larvae/adults trapped, cumulative number of larvae/adults trapped by different traps in each experimental combination were divided by total number of larvae/adults present. While, to calculate the proportion of larvae/adults trapped by different traps, the cumulative number of larvae/adults trapped by different traps in each experimental combination is multiplied by total mean efficiency of larvae/adults and then divided by total number of larvae/adults present.

Colour change was measured by using Colour Reader CR-10 (Konica Minolta Sensing Inc.) with the equation given by Gnanasekharan et al. (1992) and weight loss was calculated using the count and weight method given by Adams and Schulten (1978) for each experimental combination were recorded at regular interval of 15 days for a period of 6 months. The regression coefficients were estimated through least square method. The adequacy of fitted model was tested through the analysis of variance showing lack of fit and coefficient of correlation (R^2). The values of various responses were related to the coded variables by a second degree polynomial using the equation- $Y= b_0+ b_1x_1 + b_2x_2 + b_{12}x_1x_2+ b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_{12} + b_{22}x_{22} + b_{33}x_{32}$. Optimization of mechanical exclusion devices in different combinations to trap maximum test insect were carried out using the Box Behnken design of response surface methodology (Design-Expert software version 8.0.3.1, Stat-Ease Inc., Minneapolis, U.S.A) (Box and Behnken, 1960) with the target of finding the levels of independent variables viz. number and type of traps based on the responses of maximum trapping of insect (adults and larvae) and minimum change in colour and weight loss.

RESULTS AND DISCUSSION

The results related to application of different traps in different combinations against *T. granarium* in wheat showed that the mean maximum trapping efficiency recorded in T_2 (53.67) for larvae (A: B: C; 12.66, 63.0 and 24.24) and (66.94) for adults (A: B: C; 6.94, 75.61 and 17.46) when the total number of 8 traps were placed having combination of 1:5:2. While, minimum trapping efficiency was found in treatment T_4 (43.18) for larvae (A:B:C; 52.28, 19.39 and 28.33 proportion of total) and (53.67) for adults (A:B:C; 43.85, 18.64 and 37.52) when a total number of 6 traps were placed having combination of 3:1:2 (Table 1). The results showed that the two in one trap having 3 mm perforation was more effective. The efficiency of traps was greatly influenced by the number and type of traps, irrespective of their combinations. Present resutls corroborate with those of Rajesh et al.(2015) who recorded high frequency of detection of *Lasioderma serricorne* with higher



Fig. 1a. Probe trap



b. Two in one trap (3 mm)



c. Two in one trap (2 mm)

Table 1. Effect of different traps and their combinations for trapping *T. granarium* in wheat

Treat-ments	Combination of different traps A:B:C	Larvae trapped				Adults trapped				Colour change*	Weight loss* (%)
		Total mean efficiency* (%)	Proportion of total			Total mean efficiency* (%)	Proportion of total				
			A	B	C		A	B	C		
T_1	3:3:1	48.50	42.69	43.31	14.00	57.78	45.67	42.32	12.02	2.71	8.34
T_2	1:5:2	56.05	12.66	63.00	24.34	66.94	6.94	75.61	17.46	1.34	4.94
T_3	3:3:3	48.49	34.45	34.66	30.89	59.23	32.99	35.76	31.24	1.26	4.63
T_4	3:1:2	43.18	52.28	19.39	28.33	53.67	43.85	18.64	37.52	2.42	7.82
T_5	1:3:1	54.58	24.80	50.20	25.00	64.89	25.91	53.23	20.86	2.59	9.41
T_6	1:3:3	52.57	15.46	42.54	42.00	64.72	14.15	46.13	39.72	2.63	8.01
T_7	2:1:1	44.55	52.99	20.01	27.00	54.42	56.98	23.48	19.54	2.73	10.56
T_8	2:3:2	50.53	26.07	42.82	31.11	61.25	30.38	46.92	22.70	2.38	9.32
T_9	1:1:2	48.13	29.96	22.16	47.88	60.50	27.40	31.36	41.24	2.81	11.24
T_{10}	2:1:3	45.55	33.24	12.54	54.22	57.72	32.49	24.10	43.41	1.96	7.03
T_{11}	2:3:2	50.54	25.10	45.45	29.45	61.25	32.45	48.51	19.04	2.41	8.45
T_{12}	2:5:1	54.27	21.29	67.07	11.64	63.90	22.31	71.25	6.44	1.49	5.01
T_{13}	2:5:3	52.93	23.24	46.76	30.00	63.98	17.39	55.29	27.31	1.17	4.32
T_{14}	2:3:2	50.53	24.30	42.49	33.21	61.25	28.54	43.50	27.96	2.89	8.89
T_{15}	3:5:2	51.50	23.24	52.82	23.94	61.55	27.50	52.84	19.66	1.15	3.54

Where A-probe trap, B- Two in one trap with 3 mm perforations, C-Two in one trap with 2 mm perforations; * based on 6 month storage

number of traps, whereas the number of insect catches in wheat bag stack increased with number of traps (Hategekimana et al., 2013).

The regression equation of the fitted quadratic model for TMTEL, TMTEA are 47.57-4.46 A+ 4.81 B+0.29 C+0.05 AB +0.50 AC -0.29 BC+0.21 A^2-0.37 B^2-0.18 C^2 and 59.01-6.08 A+ 4.19 B + 1.99 C+0.18 AB +0.41 AC -0.40 BC+0.41 A^2-0.31 B^2-0.25 C^2, respectively. While, the regression equation of the fitted quadratic model for trapping larvae/ adults by different traps i.e. by probe trap (A) for larvae is 67.09 +10.49 A- 13.78 B-27.81 C-1.47 AB +0.28 AC +2.71 BC+0.52 A^2 +0.96 B^2 +3.67 C^2 and for adult is 53.38 + 21.66 A- 10.27 B -23.80 C+ 0.51 AB -0.23 AC +2.45 BC-3.32 A^2 -0.18 B^2 +2.55 C^2; by two in one trap (3mm) (B) for larvae is 0.94-3.87 A+ 22.53 B +7.11 C-0.93 AB -0.25 AC -1.60 BC+ 0.92 A^2 -1.29 B^2 -1.82 C^2 and for adult is 38.42-19.79 A- 8.12 B + 15.43 C+ 0.74 AB +0.09 AC -0.38 BC+3.76 A^2 +0.49 B^2 -1.03 C^2; by two in one trap (2mm) (C) for larvae is 31.97- 6.63 A- 8.75 B + 20.69 C+ 2.39 AB -0.027 AC -1.11 BC-1.44 A^2 +0.33 B^2 -1.85 C^2 and for adult is 8.16-1.86 A+18.39 B + 8.39 C- 1.26 AB + 0.14AC -2.07 BC-0.43 A^2 -0.32 B^2 -1.52 C^2. These regression models were highly significant ($p< 0.0001$, $R^2> 0.9436$) for all the tested parameters, with none showing lack of fit ($p > 0.01$), indicating that all the second-order polynomial models correlated well with the experimental data. It is apparent that linear terms of probe trap and two in one trap with 3 mm perforations ($p<0.01$) had significant effect on total mean efficiency of larvae trapped whereas, all the three traps witnessed significant effect on total mean efficiency of adults trapped (Table 2). Total mean efficiency for both larvae and adults trapped were highly influenced by number of traps i.e. two in one trap with 3 mm perforation followed by 2 mm perforation in comparison to probe trap recording higher total mean efficiency of larvae/ adults trapped for the experimental combination with more number of type B and C traps (Table 3). Thus, based on trapping efficiency, these traps can also be used as early warning tool as suggested by Duarte et al. (2021).

Colour change of wheat grains over a period of six months ranged from 1.15 (T_{15}; 3:5:2) to 2.81 (T_9; 1:1:2), irrespective of experimental combination witnessing positive relationship with type and number of traps installed. The quadratic model fitted for colour change was found to be significant with non-significant lack of fit (Table 3). The regression equation of the fitted quadratic model for colour change is 1.26 + 0.78 A + 0.36 B + 0.97 C+0.025 AB - 0.37 AC + 0.056 BC-0.085 A^2 - 0.136 B^2 -0.177 C^2 ; R^2 for the model fitted was found to be 0.95 for stored wheat grain after six month of storage. It is apparent that linear terms of type of traps (A, B & C) had significant effect ($p<0.01$) on colour change witnessing higher effect of two in one trap with 3mm perforations irrespective of the experimental combinations (Table 1, 2). Among the quadratic and

Table 2. ANOVA table of response surface quadratic model of use of TNAU traps for trapping *T. granarium* in wheat

Source	F-value									
	Larvae trapped				Adults trapped				Colour change	Weight loss
	Total mean efficiency (%)	Proportion of total			Total mean efficiency (%)	Proportion of total				
		A	B	C		A	B	C		
Model	108.74*	176.38*	69.43*	22.89*	119.30*	37.89*	53.25*	9.29*	10.95*	38.07*
A	237.51*	487.04*	16.99*	26.49*	406.85*	111.48*	52.00*	2.30	7.08**	46.01*
B	683.04*	775.28*	535.04*	68.26*	596.77*	146.17*	399.81*	32.57*	48.09*	190.03*
C	3.42	125.20*	42.99*	94.58*	14.34*	56.55*	13.57*	44.52*	13.21*	46.60*
AB	0.20	27.57*	2.43	10.98**	2.74	0.66	3.26	0.45	0.17	4.37
AC	4.92	0.24	0.043	3.62E-004	3.47	0.03	9.41E-003	1.68E-003	9.38**	5.71
BC	6.73**	94.20*	7.29**	2.35	13.70**	14.94*	8.87**	0.12	0.86	8.64**
A^2	0.82	0.80	0.55	0.92	3.24	6.36**	0.090	2.71	0.45	5.09
B^2	40.70*	44.00*	17.41*	0.75	30.12*	0.29	0.76	0.75	18.54*	32.55*
C^2	0.57	39.88*	2.18	1.51	1.24	3.74	1.10	0.21	1.97	8.25**
Lack of Fit	0.64	1.98	2.91	3.25	4.38	2.13	1.30	0.93	0.54	1.39
R^2	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.94	0.95	0.99
C V (%)	0.90	3.80	5.89	9.57	0.71	8.54	6.24	17.05	11.42	6.50

Where A-probe trap, B- Two in one trap with 3 mm perforations, C-Two in one trap with 2 mm perforations, *significant at 1% level and ** significant at 5% level

product terms, the product term (AC) and quadratic term (B^2) showed significant effect ($p<0.01$). Among the three traps, higher effectiveness of two in one trap with 3 mm perforation followed by 2 mm perforation in comparison to probe trap resulted in lesser colour change for the experimental combination with more number of type B and C traps (Table 1). The trapping efficiency was observed to be directly affecting the colour change with higher values in the samples with lower trapping efficiency or higher population of *T. granarium*. These results derive support from Myers and Hagstrum (2012) that presence of *T. granarium* causes contamination of grains with their body parts and cast skins leads to change in colour of grains.

The total weight loss ranged from 3.54 (T_{15}; 3:5:2) to 11.24 (T_9; 1:1:2); the quadratic model fitted for weight loss was significant ($p=0.01$; F-38.07; R^2 0.9856 (Table 2). The model clearly depicts the negative effect of trap A and B whereas positive effect of trap C on weight loss. Previous studies also reported that weight loss caused by *T. granarium* was 5-30% and up to 73% in extreme cases (Anonymous, 2005). It is apparent that linear terms of type of traps (A, B & C) had significant effect ($p<0.01$) on weight loss with two in one trap- 3 mm perforations whereas the probe trap and two in one trap with 2 mm perforations were at par, irrespective of the experimental combinations (Table 2). The regression equation of the fitted quadratic model is 0.02 – 1.51 A- 0.24 B + 1.81 C+0.25 AB -0.58 AC +0.36 BC-0.57 A^2 -0.36 B^2 -0.72 C^2. The product term (BC) and quadratic terms (B^2 and C^2) showed significant effect. Among the three traps, higher effectiveness of two in one trap with 3 mm perforation followed by 2mm perforation in comparison to probe trap was resulting in lower weight loss for the experimental combinations with more number of type B and C traps (Table 1). Further, trapping efficiency showed direct effect on weight loss as lower trapping efficiency resulted in higher weight loss. This might be due to the fact that higher population of *T. granarium* in the sample causes contamination of wheat grains resulting in powder formation causing weight loss (Demis and Yenewa, 2022). The results obtained were similar to those of Ahmedani et al. (2011).

The optimum values of number and type of different traps based on responses of trapping of larvae and adults, colour change and weight loss of wheat grains is presented in Table 3. The number and type of traps for trapping larvae and adults were optimized using numerical optimization technique for maximum trapping, and minimum change in colour and weight loss. Equal importance was given to all the parameters and responses. The zone of optimization for larval

Table 3. Optimization of mechanical exclusion devices for trapping *T. granarium* in stored wheat

Type of trap	Goal	Lower limit		Upper limit		Importance	Calculated value		Desirability	
		Larvae trapping	Adults trapping	Larvae trapping	Adults trapping		Larvae trapping	Adults trapping	Larvae trapping	Adults trapping
Probe trap	in range	1	1	3	3	3	3	2		
Two-in-one trap (3 mm perforations)	in range	1	1	5	5	3	4	5		
Two-in-one trap (2 mm perforations)	in range	1	1	3	3	3	3	3	0.61	0.63
		Response					Predicted value			
Total mean trapping efficiency (%)	Maximize	43.18	53.67	56.05	66.94	3	50.22	63.59		
Probe trapping efficiency (Proportion of total)	Maximize	12.66	6.94	52.99	56.98	3	30.62	19.42		
Two-in-one trap (3 mm perforations) trapping efficiency (Proportion of total)	Maximize	12.54	18.64	67.07	75.61	3	39.15	55.64		
Two-in-one trap (2mm perforations) trapping efficiency (Proportion of total)	Maximize	11.64	6.44	54.22	43.41	3	30.23	24.94		
Colour change	Minimize	1.15	1.15	2.89	2.89	3	1.07	1.05		
Weight loss (%)	Minimize	3.54	3.54	11.24	11.24	3	3.88	3.95		

trapping of *T. granarium* depicts that the number and type of traps; probe trap, two-in- one trap with 3 mm perforations and two- in- one trap with 2mm perforations were 3, 4 and 3, respectively recording optimized predicted values i.e. 50.22% total mean trapping efficiency, 1.07 of colour change and 3.88% weight loss with desirability of 0.61. For adult trapping, the optimal type and number of mechanical exclusion devices i.e. probe trap, two-in- one trap with 3 mm perforations and two- in- one trap with 2 mm perforations obtained were 2, 5 and 3, respectively. The corresponding values of responses obtained were 63.59% of total mean trapping efficiency, 1.05 of colour change and 3.95% weight loss with desirability of 0.63 (Table 3); thus, RSM was effective for optimizing mechanical exclusion devices. Numerical technique, in connection with RSM, was used to aid in identifying optimal conditions; these were experimentally verified and proved to be adequately reproducible. Thus, the mechanical exclusion devices used in the study could exploit the wandering behaviour of the insects and help in timely detection in stored produce leading to timely control.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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DISSIPATION AND PERSISTENCE OF IMIDACLOPRID USED AS SEED TREATMENT IN PEAS

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ABSTRACT

Dissipation and persistence study of imidacloprid on peas as seed treatment revealed presence of 6.47 and 9.92 mg kg^{-1} residues on green pea leaves at a dose of 1.8 g and 3.6 g a.i. per kg of seed, respectively after 19 days of treatment. Residues of imidacloprid dissipated below limit of quantification of 0.01 mg kg^{-1} after 49 and 54 days of treatment. Residues of imidacloprid were found below limit of quantification of 0.01 mg kg^{-1} in immature pod with succulent seeds, succulent seeds (shelled), mature pod with seeds, mature seeds (shelled), soil and dry fodder (without root and pod).

Key words: Imidacloprid, seed treatment, peas, residues, persistence, leaves, seed, mature pod, fodder, QuEChERS method

Neonicotinoids have a novel mode of action by acting as agonists at the insect nicotinic acetylcholine receptor (Tomizawa and Casida, 2011), these have selective-toxicity (Jeschke et al., 2011), and have systemic action (Stoner and Eitzer, 2012). Neonicotinoid seed treatments have shown long-lasting residual activity against aphids (Zhang et al., 2016a,b) and thrips in soybean and groundnut (Reisig et al., 2012; Zidan, 2012; Nataraja et al., 2016). Peas is an ancient cultivated leguminous crop (Dahl et al., 2012), and it is attacked by many pests, out of which aphids, thrips, leaf miner and pea stem fly are the most destructive (Anonymous 2019). Pea aphid *Acyrthosiphon pisum* is a serious pest causing losses of up to 35.7% (Barlow et al., 1977); Bhatnagar 1996). Presence of pesticide residues on agricultural products is a cause of concern, and emphasis has been given to resolve problems associated with the pesticide residues, indiscriminate and irrational use of pesticides. Presence of pesticide residues in food commodities is of consumer health concern and many studies have reported presence of imidacloprid residues (Chen et al., 2014; Shi et al., 2019). Since, the magnitude of pesticide residues in the vegetable commodities at harvest is a cause of concern in many of the occasions, the present study focused on evaluating the persistence and dissipation of imidacloprid applied as seed treatment in pea crop.

MATERIALS AND METHODS

Field experiment was conducted during rabi 2019-20 at the Entomological Research Farm, Punjab Agricultural University, Ludhiana following standard agronomic practices (Anonymous, 2019). Pea (variety Punjab 89) seeds were treated with an aqueous slurry of imidacloprid 600 FS at recommended dose (1.8 g a.i.kg^{-1} seed) and double the recommended dose (3.6 g a.i.kg^{-1} seed). Green peas seeds were put in polythene bags separately for each treatment and thoroughly mixed with slurry made from pesticide and water with volume of 12 ml/ kg seed (pesticide + water) and then dried in shade before sowing. Urea and superphosphate fertilizers were applied as per the practices recommended for cultivation of peas in Punjab (Anonymous, 2019). Pea leaf samples were collected randomly from each plot on 19th day of sowing followed by 20, 22, 24, 26, 29, 34, 39, 44, 49, 54 days after sowing. Samples of immature pods and succulent seed (shelled) samples were also collected at the time of first picking i.e. 110 days after sowing, while mature pods with seeds, mature seeds (shelled), dry fodder (without root and pod) and soil samples were collected at the time of harvest (128 days after sowing). Immature pods with succulent seeds, succulent seeds (shelled), mature pods with seeds, mature seeds (shelled), dry fodder (without root and pod) and soil samples were prepared following QuEChERS method for the determination of residues of imidacloprid by the method suggested by Akoijam et al. (2015) with slight modifications.

A sub sample of 15 g was weighed into a 50 ml centrifuge tube and then 30 ml acetonitrile was dispensed into it while in case of dry fodder a sub sample of 5 g was weighed into a 50 ml centrifuge tube and then

20 ml acetonitrile was dispensed into it. In case of soil, 10 ml of distilled water was added in addition to 30 ml acetonitrile. The sample was homogenized using high speed homogenizer (Heidolph Silent Crusher-M®) for 3 min at 15,000 rpm. Sodium chloride (NaCl) @10± 0.1 g was added to homogenize the sample for phase separation. The contents were centrifuged at 2,500 rpm for 3 min. An aliquot of 15 ml acetonitrile layer was transferred over 10± 0.1 g sodium sulfate (Na_2SO_4) in a test tube. The acetonitrile extract was subjected to cleanup by dispersive solid phase extraction (DSPE). An aliquot of 6 ml acetonitrile was taken in a test tube containing 0.15± 0.01 g PSA sorbent, 0.90± 0.01 g anhydrous $MgSO_4$ and 0.05± 0.01g graphitic carbon black and the contents were thoroughly vortex on vortex shaker. 4 ml aliquot was taken for estimation of imidacloprid residues. Sodium chloride (NaCl) and anhydrous sodium sulfate (Na_2SO_4) was bought from Sisco Research Laboratories Pvt. Ltd., New Mumbai, India. Before use anhydrous sodium sulphate (Na_2SO_4) and anhydrous magnesium sulphate ($MgSO_4$) were activated for 4 h at 800°C in muffle furnace to remove impurities of phthalate. Primary Secondary amine (PSA) bondesil, anhydrous magnesium sulphate ($MgSO_4$) and graphitized carbon black (GCB) were procured from Agilent Technologies Ltd. Bangalore. During analysis, blank samples were also run along with sample to check the suitability of these solvents.

The estimation of imidacloprid residues was done by using high performance liquid chromatography (HPLC) system (Make: Shimadzu Corporation, Kyoto, Japan; Model: SIL 20A/20 AC) with double plunger pump, Rheodyne injector having 20 µl loop with PDA detector. The chromatographic separation was achieved on C_{18} reverse phase column (4×150mm) with a particle size of 5 µm. The mobile phase consisted of HPLC grade acetonitrile (Make: Qualichem Lab): water (30:70 v/v) with a constant flow rate of 0.3 ml/ min and UV detection set at 272 nm. The method of estimating pesticide residues was further validated in terms of linearity, efficiency, precision (repeatability), limit of detection and limit of quantification by spiking the samples at different fortification levels of imidacloprid to assess the reliability and validity of the analytical method (Akoijam et al., 2015). Samples were spiked at three different concentrations *viz*., 0.01, 0.05 and 0.1 mg kg^{-1} and imidacloprid residues were calculated by comparing the peak areas of the reference standards with that of the unknown or spiked samples run under identical working conditions of the instruments. The data was analyzed for mean and standard deviation.

RESULTS AND DISCUSSION

For the validation of the analytical method sensitivity, selectivity, calibration and recovery experiments were performed. Graphs were constructed from chromatograms by plotting the peak area of the signal response versus the concentration of the analyte to assess the linear response of imidacloprid. The control samples were fortified with standards at 0.01, 0.05, and 0.10 mg kg^{-1} concentration levels of imidacloprid standard. The residues were extracted, cleaned up as per methodology mentioned above and analyzed by using high performance liquid chromatography. The untreated control samples were also processed as above to find any interference caused by matrix/substrate and reagents. The analytical method was found to be suitable since it did not show any interference peaks at the retention time of imidacloprid and was sensitive in terms of response. The recovery percentage of imidacloprid on pea leaf and pod samples spiked at 0.01, 0.05 and 0.1 mg kg^{-1} was also found to be very suitable varying between 88.78-98.33% which was within the acceptable range. The limit of quantification (LOQ) was found to be 0.01 mg kg^{-1} and limit of detection (LOD) being 0.003 mg kg^{-1}. The data also revealed that the analytical method was reliable and valid, and on the extraction and cleanup procedures were also effective and hence could be presented as such without any correction factor. Residues of imidacloprid on green pea leaves sampled at 19th day after treatment was found to be 6.47 and 9.92 mg kg^{-1} at the dose of 1.8 and 3.6 g a.i.kg^{-1}, respectively, which decreased to5.83 and 8.71 mg kg^{-1} at 20th day after treatment with 9.89% and 12.19% reduction, respectively. Residues of imidacloprid dissipated below limit of quantification of 0.01 mg kg^{-1} at 49 and 54 days after treatment in both the tested doses. Residues of imidacloprid were below the limit of quantification of (0.01 mg kg^{-1}) in immature pod with succulent seeds, succulent seeds (shelled), mature pod with seeds, mature seeds (shelled), while the samples of soil and dry fodder (without root and pod) collected at harvest were free from imidacloprid residues (Table 1, Fig. 1).

Present results are in agreement with those of other researchers regarding dissipation rate of imidacloprid in brinjal, bengal gram and tomato. Imidacloprid residues have been reported to dissipate below the limit of quantification after 7 and 10 days in brinjal from initial deposits of 0.72 and 1.92 mg kg^{-1} following application of spinetoram at 12% + imidacloprid 12% @ 75 and 150 g a.i. ha^{-1} respectively on brinjal crop (Bhardwaj et al., 2016). Likewise in another set of

Table 1. Recovery (%) and residue estimation of imidacloprid (mg kg^{-1}) on pea crop and soil

Recovery of imidacloprid on different matrices of pea crop and soil		
Substrates	Level of fortification (mg kg^{-1})	Recovery (%)
Leaves	0.01	98.33± 3.31
	0.05	96.57± 3.47
	0.10	96.42± 2.84
Immature pods with succulent seeds	0.01	95.35± 3.56
	0.05	89.26± 2.98
	0.10	88.78± 1.82
Succulent seeds (shelled)	0.01	87.21± 3.00
	0.05	87.23± 1.93
	0.10	93.05± 2.24
Mature pods with seeds	0.01	94.92± 3.14
	0.05	91.57± 3.47
	0.10	87.99± 3.64
Mature seeds (shelled)	0.01	88.20± 3.85
	0.05	85.56± 2.95
	0.10	91.07± 3.15
Dry fodder (without root and pod)	0.01	96.52± 3.67
	0.05	94.46± 2.30
	0.10	86.49± 1.95
Soil	0.01	96.47± 3.12
	0.05	90.11± 1.26
	0.10	96.37± 1.86

Days after treatment	Residues of imidacloprid (mg kg^{-1}) after application @ 1.8 g a.i.kg^{-1} seed		3.6 g a.i.kg^{-1} seed	
	Mean± S.D.	Dissipation (%)	Mean± S.D.	Dissipation (%)
	Green pea leaves			
19	6.47± 0.23	-	9.92± 0.36	-
20	5.83± 0.35	9.89	8.71± 0.51	12.19
22	3.99± 0.10	42.50	6.32± 0.31	36.29
24	2.49± 0.38	61.51	4.25± 0.15	57.15
26	1.51± 0.11	76.66	3.17± 0.14	68.04
29	1.19± 0.13	81.60	1.90± 0.23	80.84
34	0.46± 0.03	92.89	1.28± 0.08	87.09
39	0.15± 0.04	97.68	0.44± 0.12	95.56
44	0.03± 0.01	99.53	0.07± 0.01	99.59
49	<LOQ	-	0.042± 0.00	-
54	<LOQ	-	<LOQ	-
	Immature pods with succulent seeds			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Succulent seeds (shelled)			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Mature pods with seeds			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Mature seeds (shelled)			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Dry Fodder (without root and pod)			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Soil			
110	<LOQ	<LOQ	<LOQ	<LOQ

*Limit of quantification (LOQ) = 0.01mg kg^{-1}; *Data mean of three replications

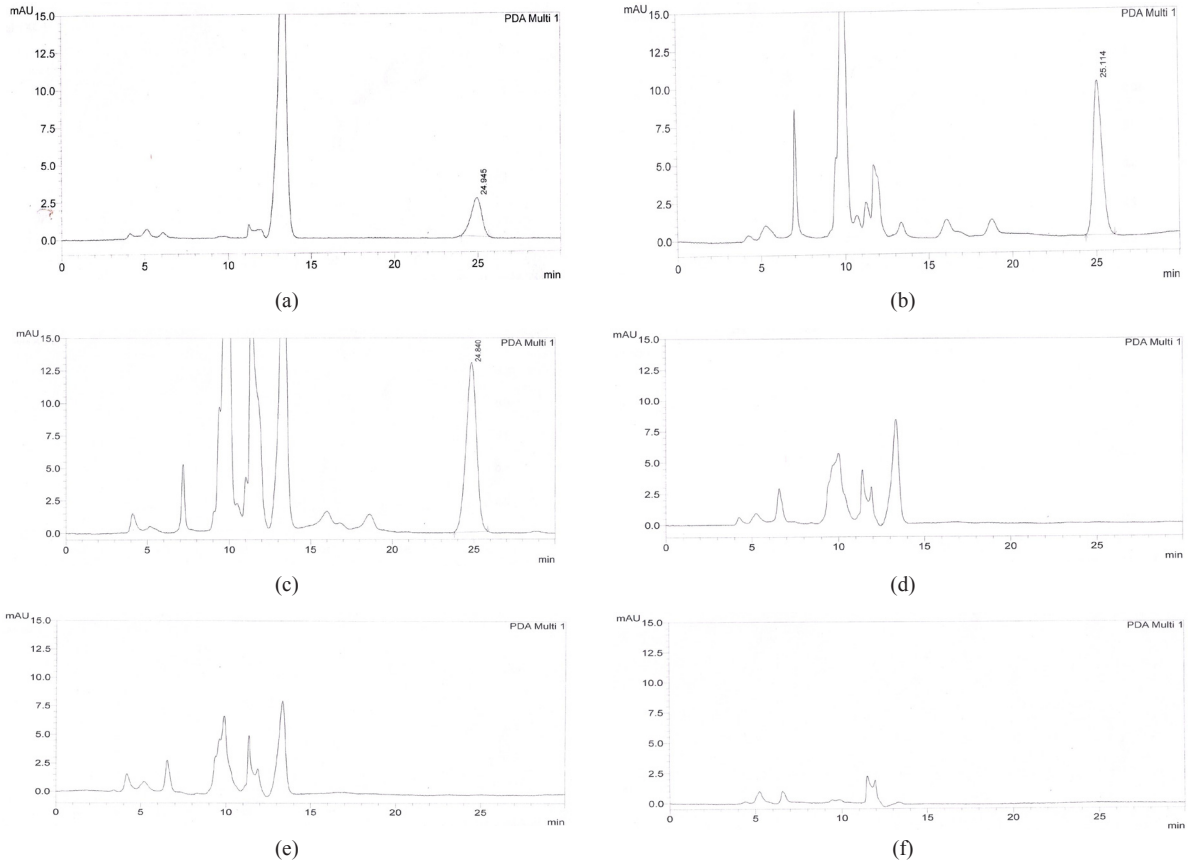


Fig. 1. Chromatograms of (a) imidacloprid standard (20 ng) (b) pea leaf sampled at 19 days after sowing (recommended dose) (c) pea leaf sampled at 19 days after sowing (double the recommended dose) (d) mature pod with seed samples (e) succulent seed samples and (f) soil samples

experiments were conducted by Gupta et al. (2005) to study the persistence of imidacloprid in gram following foliar application @ 20 and 40 g ai ha^{-1}. The results revealed that residues of imidacloprid persisted beyond 3 days but no residues were detected on 5th day and the residues were not detected in harvested seed and fodder samples. Earlier also, Romeh et al. (2009) have reported the dissipation of imidacloprid in tomato fruits by the application of imidacloprid 20 SC @ 10 g a.i. $feddan^{-1}$ (1 feddan = 4,200 m^2) and found average initial deposits of 4.22 and 1.95 mg kg^{-1} in the leaves and tomato fruits which dissipated to 0.44 and 0.075 mg kg^{-1} respectively 14 days after spraying. The variation in results on dissipation of a pesticide could be attributed to crop type, method of application, cropping season and other agroclimatic variations. In another study also, Sahoo et al. (2012) found that soil samples collected at 15 days did not show the presence of imidacloprid at their detection limit of 0.01 mg kg^{-1} after application of Solomon (β-cyfluthrin 9% + imidacloprid 21%) @ 60 and 120 g a.i. ha^{-1} in okra crop. It can be concluded that after seed treatment of peas with imidacloprid @ 1.8 and 3.6 g a.i.kg^{-1}, the initial deposits reached below the limit of quantification in pea leaves after 49 and 54 days at the respective doses and these residues were below the limit of quantification in immature pods, seeds and mature pods. Hence, seed treatment of peas with imidacloprid @ 1.8 and 3.6 g a.i.kg^{-1} seed does not result in residues above the quantification limit in pea seeds and could be integrated in IPM.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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FIRST RECORD OF MAYFLY *POVILLA* (*LANGUIDIPES*) *TAPROBANES* HUBBARD FROM KARNATAKA

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ABSTRACT

Mayflies are important as macroinvertebrates of aquatic ecosystems and are reliable bioindicators of water quality. Most mayfly nymphs feed by scraping algae from underwater rocks or by extracting food particles in the littoral and benthic zones of the water bodies. The mayfly *Povilla* (*Languidipes*) *taprobanes* originally described from Sri Lanka was observed in plastic lined ponds in coastal Karnataka. Not only, this is the first report of the species from Karnataka, but the unique case making behaviour of this species is also described for the first time. Additionally, the species was found damaging plastic tarpaulins of 750 gsm thickness used as lining for water storage in the tank by chewing. This chewing behaviour was responsible for damage of plastic sheets leading to water leakage. The possible loss caused and a need of alternate strategies to store water is discussed.

Key words: Mayflies, Polymitarcyidae, *Povilla* (*Languidipes*) *taprobanes*, plastic feeding, naiad, aquatic, water tanks, plastic lining, earthen cases, microplastics

Mayflies (Ephemeroptera), with aquatic larvae and with a short terrestrial adult life play an important role in the food chain. The group comprises of about 3200 species under 410 genera and 42 families (Bauernfeind and Soldan, 2012; Selvakumar et al., 2019), of these 390 species are from the Oriental region (Selvakumar et al., 2019). Ephemeroptera comprises of 142 species from 56 genera under 15 families (Sivaramakrishnan, 2016). Mayflies are soft-bodied paleopterans widely distributed across the Indian subcontinent. Larvae are confined to lentic and lotic freshwater habitats. Most larvae feed by scraping algae from underwater rocks and higher plants or by extracting food particles from mud. Not much is known about their biology and systematics. Only few families such as Baetidae, Ephemeridae, Heptageniidae, Leptophlebiidae and Potamanthidae have received some attention (Balachandran et al., 2011). Most species are free-living in adult stage with lifespan of only one or two days, but the aquatic naiad lasts up to a year or more. Among its families, lesser-known family Polymitarcyidae is represented by only two genera with 13 species. Among these, three species are known only by naiads. Polymitarcyidae are commonly called burrowing mayflies which burrow in rivers under rocks or in clay banks (Bouchard, 2004). Herein included is the first record of a species from the coastal Karnataka. Its behaviour that leads to the damage of plastic pond lining is described here highlighting the loss of water from such water conservation ponds.

MARTIALS AND METHODS

In February 2020, one of the authors (KV) observed declining water levels in an on-farm irrigation pond at the campus of Varanashi Farms, Adyanadka, Bantwala Taluk, Dakshina Kannada District, Karnataka, India (12.69N, 75.10E). The pond is approximately 4.7 m deep and with a capacity of 600 m^3 and lined with 750 gsm tarpaulin sheet. Careful inspection of the ponds over successive days revealed loss of water only in those ponds where the lining had not been properly stretched and had developed folds. The transfer of fishes from the leaking pond to another pond measuring 23x 17x 4.8 m also developed water leakage. Suspecting the transfer of invertebrates from the first pond, the second pond was emptied between January and February 2021 and closely examined for damage. Close examination the plastic folds revealed several soil lined burrows and earthen cases with aquatic invertebrates. Specimens of the invertebrates from the specific sites of damage were collected and identified to ascertain the cause of damage to the pond lining.

The plastic folding where the lining was damaged with punctures had small tunnels or burrows and earthen cases made of fine silt with very small fragments of the plastic lining (Fig. 1 a-c). These tunnels and cases were found to harbour larvae of mayflies (Fig. 1 d-e) and often the disturbance forced out the larvae from their resting places enabling collection. Invariably the plastic lining of the pond was damaged wherever there was an aggregation of the larvae. Samples of free-living larvae were also taken from the pond using an aquatic net. The larvae were collected in 90% ethyl alcohol from the second pond and identified to species level based on the keys provided by Hubbard (1984) and Rathinakumar et al. (2019) and also confirmed by a taxonomist specialising in Ephemeroptera (Dr. Boonsatien Boonsoong, Bangkok, Thailand). Further, efforts were also made to collect information from Mr Sebastian, Proprietor Shakthi Tarpaulin in Kerala who supply tarpaulin sheet for farmers and also people who previously encountered holes in tarpaulins installed in rain water harvesting units. The information obtained is also represented to indicate the importance of correct diagnosis.

RESULTS AND DISCUSSION

Based on the characters examined, the specimens were identified as *Povilla* (*Languidipes*) *taprobanes* Hubbard 1984. The identification characters of *Povilla (Languidipes) taprobanes* Hubbard as follows: Length: 15-17 mm, body yellowish, dark brown dorsally, streamlined with series of gills directed backwards and placed dorsally on the abdomen (Fig. 2a,c). Head yellowish with brown reticulation (Fig. 2b); frontal process moderately developed, semi-circular; mandibles brown in distal half, yellowish in basal half; mandibles lacking incisors (Fig. 2b,d); antennae yellowish white (Fig. 2d). Thorax: pronotum golden yellow; mesonotum brownish; pleura and sternum whitish. Legs white, with whitish to yellowish setae. Forelegs with scattered hairs (Fig. 2e) whereas hind legs with a row of setae on femur and tibia (Fig. 2f). Abdomen yellowish brown dorsally with paired brown irregular blotches on each segment and a medium pale stripe running across the segments; abdomen laterally yellow and whitish ventrally; gill 1 uniramous (Fig. 2 g, h); gills 2-7 biramous (Fig. 3b), white, median line and fringe lined with black. In males, the cerci are longer with fewer spines than median caudal filament. In females (Fig. 3a) the median caudal filament is longer than the cerci, and all three terminal filaments are heavily spinose.

Material examined: Mature naiads: Farm-pond, Varanashi Farms, Adyanadka, Bantwala Taluk, Dakshina Kannada District (12.69N; 75.10E). Coll. Kalleshwaraswamy CM. 28.i.2021 and Sachin G. Pai on 17.ii.2021.

Povilla (*Languidipes*) *taprobanes* was originally described from Sri Lanka (Hubbard, 1984) and later observed from Tamil Nadu (Rathinakumar et al., 2019). Herein its occurrence from Karnataka is reported for the first time. The larvae when disturbed were observed to come out of the cases made of mostly silt and sand with tiny fragments of plastic (Fig. 4 a, b). The holes were found with cases on the plastic sheets folding, strongly implicating the larvae in the damage to the lining. It is presumed that the damage could have been caused due to larvae burrowing in the fold, and chewing or clawing through the plastic lining to build earthen cases. It is also possible that the larvae used the folds as an 'enemy free space' to hide from their natural predators in the aquatic habitat (Gallitelli et al., 2021). The loss appears to be though less, considering that large number of farmers following this system of water conservation, where plastic sheets are laid on the dug pits of different sizes and water is stored the damage can be critical. Hence, there is a need of ascertaining case making and chewing behaviour. At this juncture, it is not clear, whether there is any ingestion of plastic by the mayflies. Recently, the ingestion of microplastics by Ephemeridae (Baetidae, Heptageniidae) was proved in riverine habitats (Windsor et al., 2019).

To prevent the seepage and other losses from the water body, ponds should be lined with suitable material. Various design and materials are being used to prevent seepage from the farm ponds. Plastic can be used very effectively in lining the ponds. But, lining the ponds with plastics as a barrier material, sufficient care is required in selecting the proper material and laying of plastics material and saving it from the damage (Kumar and Singh, 2010). Polyethylene lining is the cheapest among all the lining materials, which are conventionally being used. Without any lining the usual seepage loss is 18.56 l/ hr/ m^2 indicating the need of lining material (Deepika and Rao, 2018). They also reported that with the polythene sheet lining, seepage loss was reduced to 0.32 litre/ht/m^2 indicating which was better than the cement+ soil plastering indicating the importance of polythene sheet lining. However, this viable strategy under risk due to mayfly damage.

A short survey in Kerala state brought out one

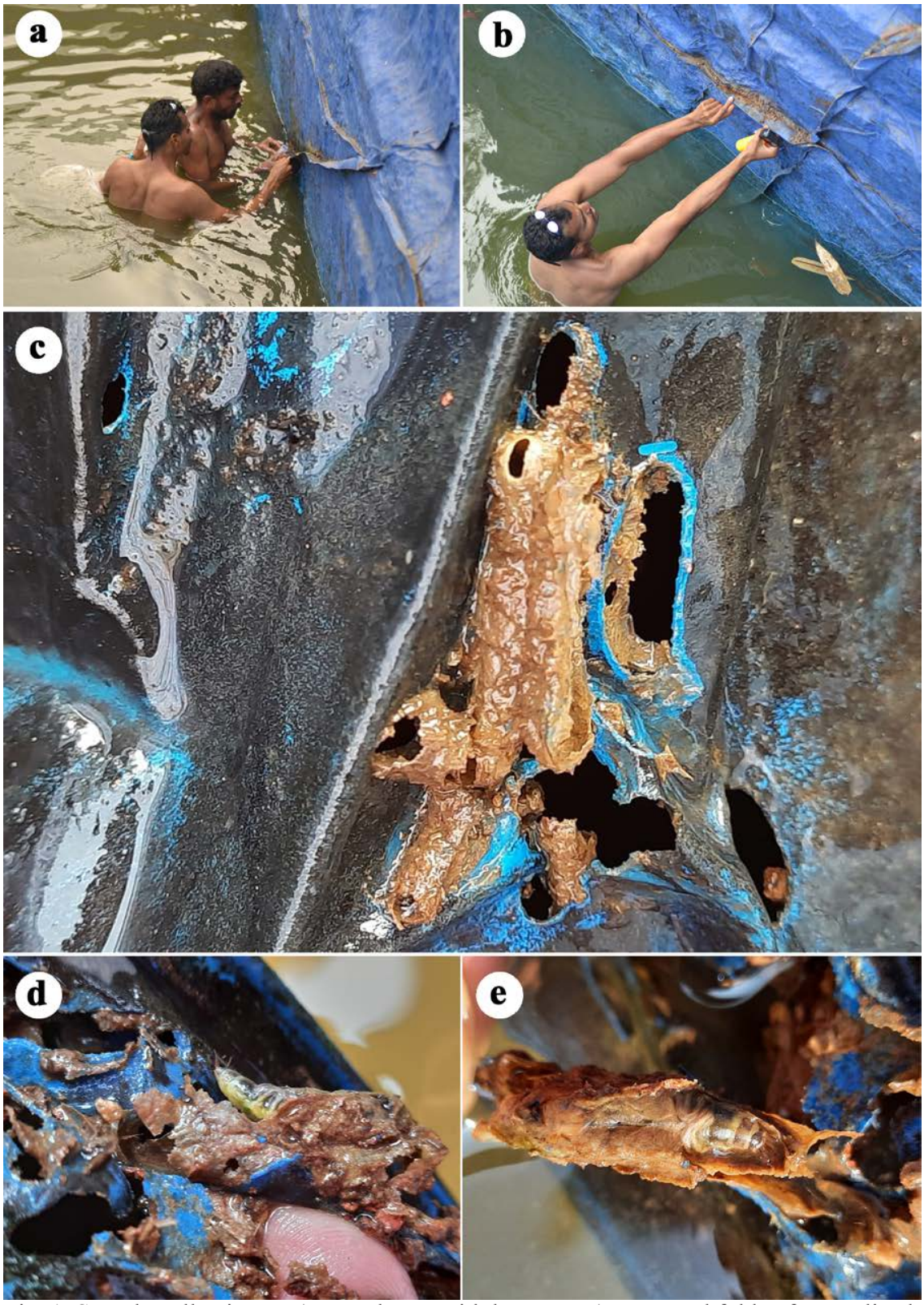


Fig. 1. Sample collection; a-c) Larval case with larvae; c-e) Damaged folds of tarpaulin sheet

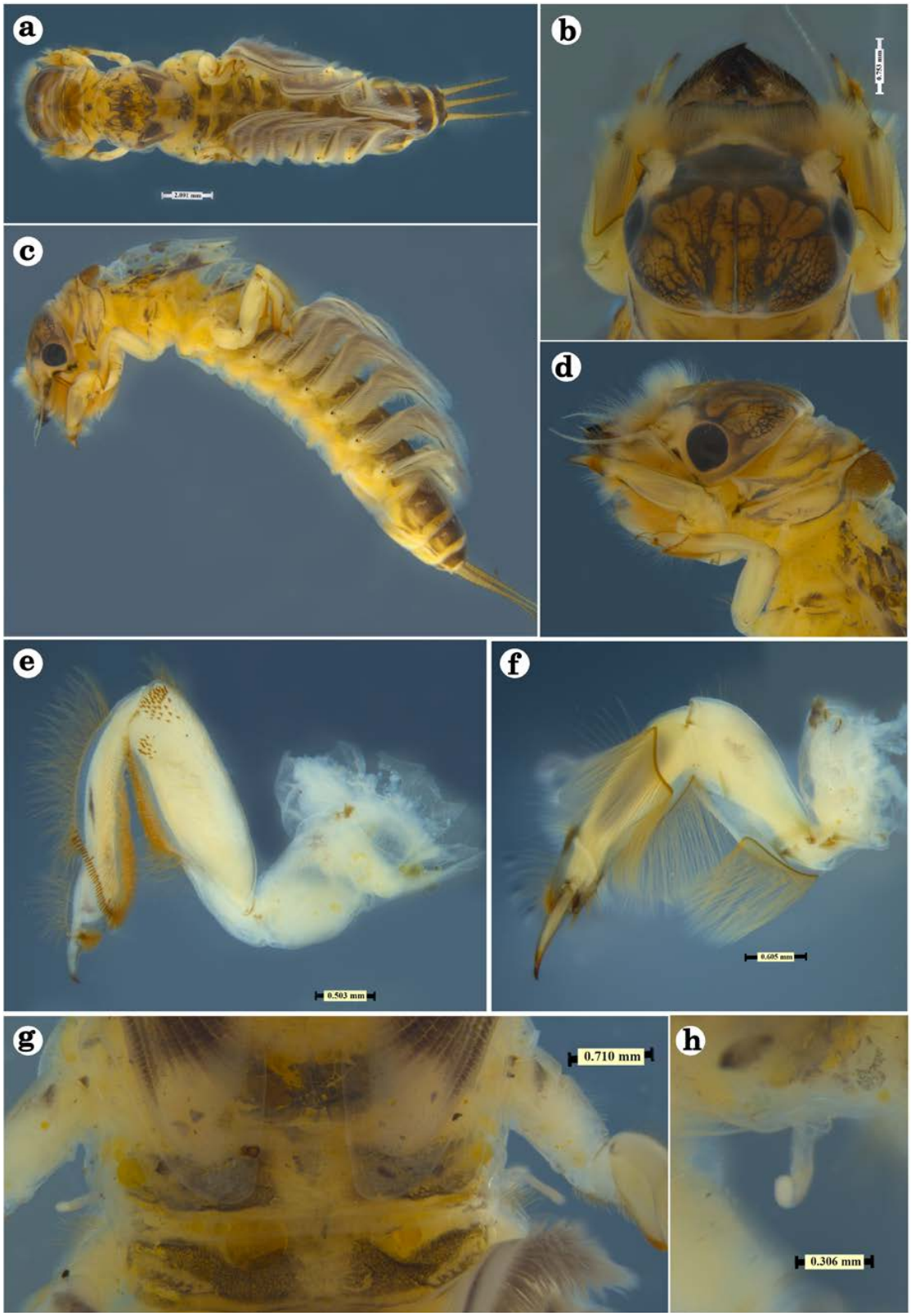


Fig. 2. *P. (L.) taprobanes*- larvae, dorsal view: a. body; b. head; Lateral view: c. larvae; d. head; e. Hind leg; f. Fore leg; g. Dorsal view, first abdominal segment; h. First abdominal gill (uniramous gill)

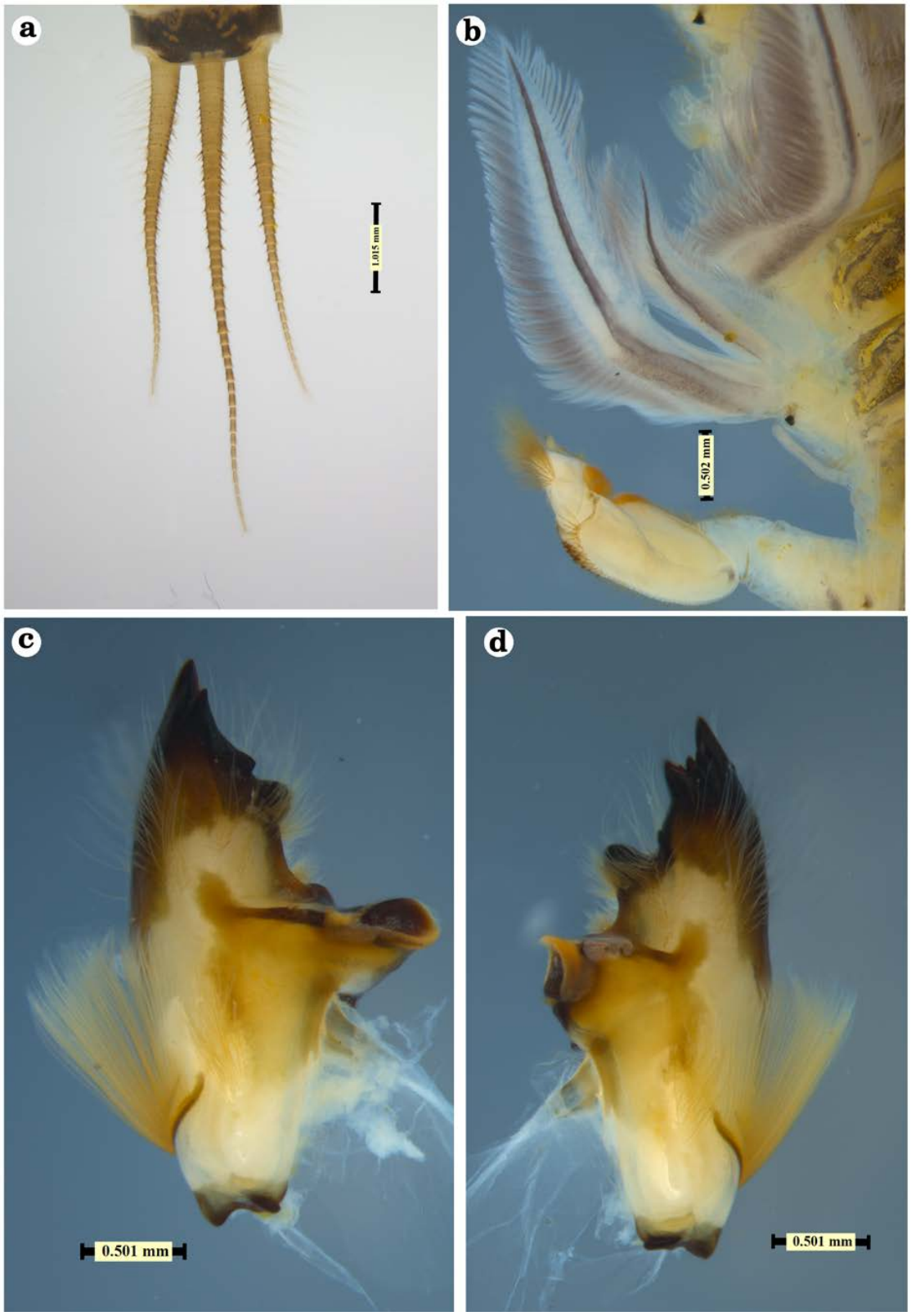


Fig. 3. *P. (L.) taprobanes* larvae; a. Cerci; b. Biramous gill of abdomen; c. Left mandible; d. Right mandible

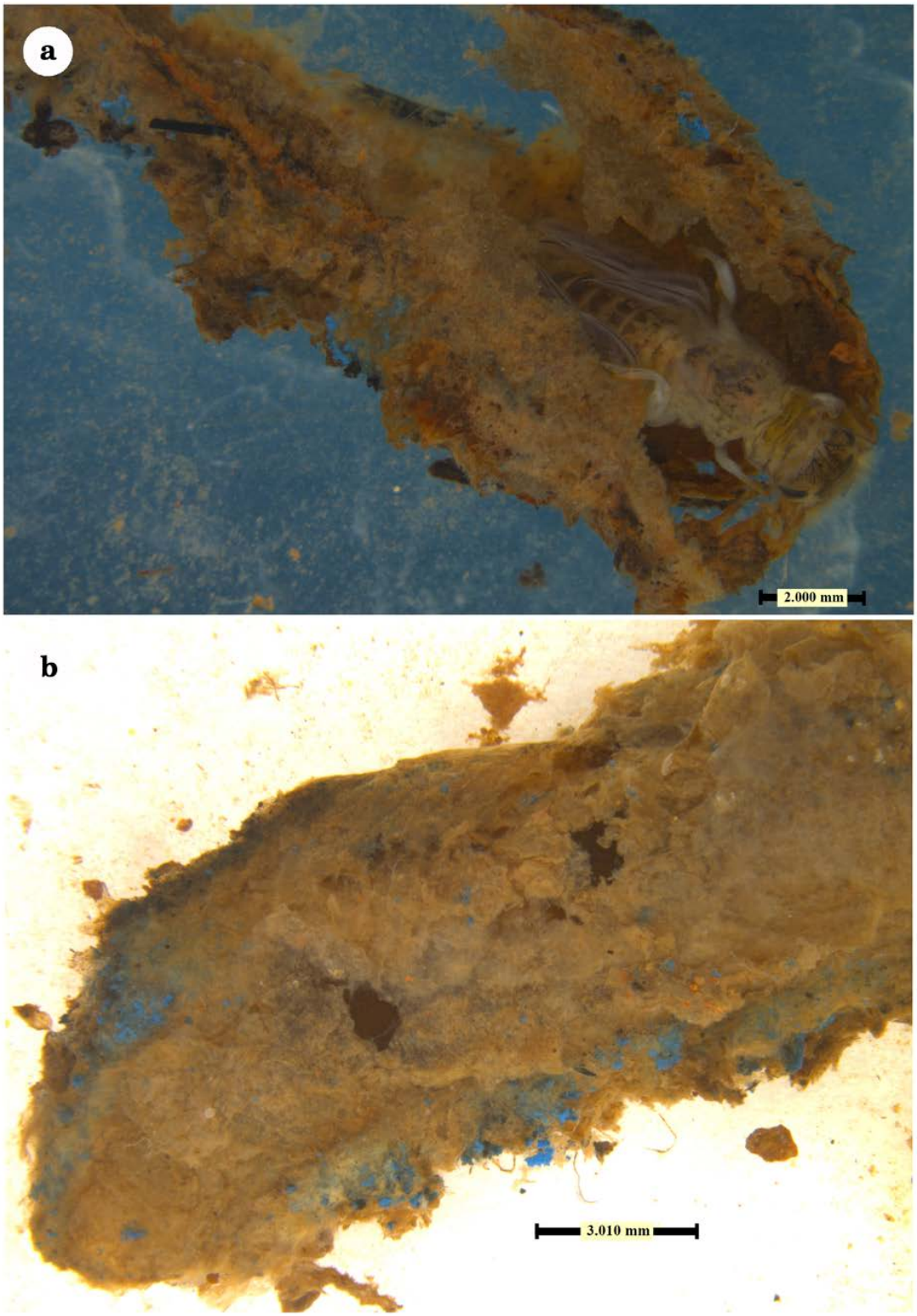


Fig. 4. *P. (L.) taprobanes* larvae; a. Larval case with naiad; b. Larval case with plastic particles

interesting information with respect to the tarpaulin damage installed in tanks for water conservation. Mr. Sebastian who was the proprietor of Shakthi tarpaulins, Payyanur, Kerala supplied polythene sheet to many farmers and organisations. The supplier regularly encountered complaints from the buyers and one organisation named, Chinmaya Mission, filed a lawsuit at the consumer court and the trial ran for around three years. The supplier got help from the officials of the previous case who produced the damage samples along with the insect. Finally, they came to terms that a new tarpaulin will be provided at the lowest cost. However, efforts made to identify the farmers who are now encountering this problem did not yield any result as many switched over to concrete lining. There is a need of observing the distribution of this species in India and damage caused if any to the plastic lining of water ponds.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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FEEDING GUILD STRUCTURE OF BIRDS AT KESHOPUR CHHAMB WETLAND, GURDASPUR

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ABSTRACT

To achieve a comprehensive understanding of avian feeding guild structure the present study was conducted at Keshopur wetland Ramsar site, district Gurdaspur, Punjab, India. A total of 121 species of birds belonging to 19 orders and 47 families were observed. Direct observation techniques and focal sampling methods were used to study their foraging behaviour. Seven major feeding guilds ie., carnivorous, insectivorous, omnivorous, granivorous, herbivorous, frugivorous and nectarivorous were found. Maximum number of species were of carnivorous guild (28%) followed by insectivorous (21%). Cattle egret and red-wattled lapwing, members of carnivorous guild were observed to feed on insects also, hence placed in multiple guilds. Northern shoveler, eurasian coots and purple moorhen were seen foraging in association with each other. The large number of insectivorous birds depicts the presence of rich number of insects. The presence of birds belonging to seven foraging guilds highlight the significance of Keshopur wetland in providing a rich feeding ground to both migratory as well as resident birds.

Key words: Punjab, Keshopur wetland, Ramsar, birds, feeding guilds, carnivorous, insectivorous, omnivorous, insects, migratory and resident birds, foraging

About 10,000 species of birds are known from the world of which Indian birds include 1346 species (Denis, 2016). Punjab state is rich in birds amongst faunal diversity (MOEF, 2019). The birds which depend on wetlands for procreation, roosting and food directly or indirectly are called wetland birds (Harisha, 2016). Wetlands are areas of land that are permanently or temporarily covered with water and are counted among most distinctive and high yielding ecosystems (Rajasekar et al*.,* 2008). About 10% of the bird species globally rely entirely on wetlands, with about 20% utilizing them for foraging, resting, breeding and overwintering (Rannestad et al*.*, 2015; Kačergytė et al*.,* 2021). In India, about 58.2 million ha fall under wetlands, and 71878.5 ha are in Punjab (Prasad et al*.,* 2002). In wetlands, the diversity and abundance of birds depend on vegetation and food availability (Rathore and Padate, 2017; Veeramani et al., 2018). Of the 37 Ramsar sites in India, six are located in Punjab, and one of them is Keshopur Chhamb Community reserve, in district Gurdaspur. This wetland has been declared as the first community reserve of India and announced as National wetland. Due to its conversion into productive agricultural land and fish farms by the Drainage Department of Government, the wetland area has been reduced. Due to human disturbance wetland birds get reduced, and wetlands in India cope with enormous anthropogenic pressures, with bird community getting influenced (Anderson et al*.*, 2019; Ringam and Shafi'u 2019). Eventhough these sites are transformed, still provide suitable environment for many birds (Bal and Dua, 2010). In order to draft conservation plans it is essential to document the bird species (Jamwal et al., 2017). Hence, the present study on the avian feeding guilds in Keshopur Chhamb Community reserve.

MATERIALS AND METHODS

Keshopur wetland is a freshwater ecosystem (32˚05'16.3"N, 75˚24'24.2"E, 245 masl, 344 ha) adjacent to the town of Gurdaspur, District Gurdaspur, Punjab. The study area was divided into three sites (Site I, II and III) for taking observations from July 2019 to June 2020. Site I consisted of trees, shrubs, herbs, grasses, aquatic plants, and climbers, and located near the road surrounded by agricultural fields. Site II mainly consisted of small vegetation including herbs, shrubs, climbers, grasses, aquatic plants, some trees and fish ponds surrounded by agricultural fields. Site III was a separate patch consisted of herbs, shrubs, grasses, large number of aquatic plants and bamboo trees.

Point count method was used to study and record the birds visiting at different sites from the watchtowers weekly for 90 min between 6:00-9:00 a.m. and 4:00-7:00 p.m. Approximately 10-15 min were spent at each point to avoid repeated counting of same bird individual.

Identification of birds residing and visiting selected sites was done on the basis of visual observations on their morphological features like shape, size, color of beak, feathers, wings, eyes, feet, legs and other parts of body by using binocular and comparing them with those described by (Ali, 2002). Species were classified into resident (R), winter migrant (WM) and summer migrant (SM) and IUCN status was also assigned as per IUCN list of endangered species. To study the foraging behavior of birds, direct observation techniques and focal sampling method (Norazlimi and Ramli, 2015) were used. In direct observation, the feeding activities and techniques of birds were directly observed using binoculars. While in focal sampling method, an individual from a flock of birds was selected and all the foraging activities were recorded using binoculars for a minimum of 30 min duration. The actively foraging birds involving those searching for prey or swallowing the prey were selected. Digital single-lens reflex (DSLR) camera (Nikon D3300) was used for photography and binocular (Vanguard FR-1050W 10x 50) was used for observation of birds.

RESULTS AND DISCUSSION

Total 121 species of birds belonging to 19 orders and 47 families were observed (Table 1), with maximum of 38 belonging to order Passeriformes. The birds were seen to have seven major feeding guilds (Fig. 1). About 34 species were observed feeding on animal matter like frogs, invertebrates, fishes and reptiles. The cormorants and pond heron were observed to feed mainly on fishes. These species were also seen competing for foraging zones at Kole wetland, Kerala (Paleeri and Jayson, 2018). Most of the small wading birds like cattle egret and red-wattled lapwing were recorded finding food in shallow water because catching of prey is easy in shallow water and also there is presence of more number of preys on the edges. The larger wading birds like grey heron and purple heron were seen standing and waiting to capture the prey. Grey herons are known for choosing bigger fish and neglecting smaller ones (Jakubas and Manikowska, 2011). The main food for black crowned night heron was fish while amphibians and crustaceans were secondary preferences. Mostly asian openbills were recorded feeding upon mollusks in the morning and evening only as also noted by Paleeri and Jayson (2018) at Kole wetlands (Thrissur) Kerala.

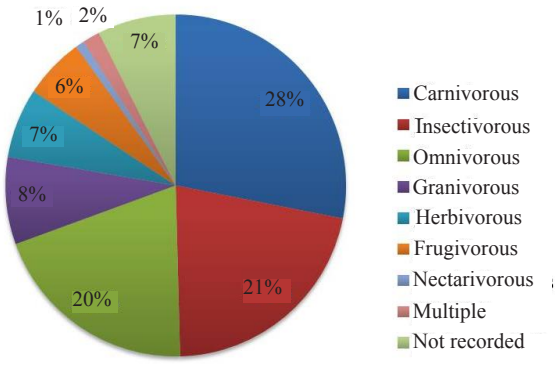


Fig. 1. Feeding guilds of birds

The presence of 26 insectivorous birds indicates the presence of insects. These birds perform an essential role in biocontrol of many insect pests growing in horticulture and agriculture (Thakur et al., 2010). The Asian paradise flycatcher feeds on flies, butterflies, dragonflies, and same behaviour was recorded by Das and Adhikari (2019). Insects were the key source of food for black-winged stilt chicks, with maximum of Diptera (Ueng et al., 2009), with these found in muddy areas. Long tailed shrikes were recorded mostly feeding on large insects and lizards (Abed and Salim, 2019). In omnivorous guild, common moorhen were feeding in groups as well as alone on parts of vegetation and invertebrates. Wallau et al. (2010) also observed the omnivorous behavior of the common moorhen at Rio Grande, Brazil. Asian pied starlings were seen foraging in alliance with other birds such as common myna, house crow, red-wattled lapwing as also reported by Sethi and Kumar (2018). The northern shovelers were seen feeding on vegetation in shallow waters due to the availability of crustaceans, benthic preys and insect larvae. The association of northern shoveler and eurasian coots was observed now corroborates with the results of Kallander (2005). Moreover, Eurasian coots were also seen feeding with purple moorhens showing similar behavior, walking and feeding, as also observed by Menon (2008). Species with a more active foraging mode were more prone to predation and thus can enter flocks more often (Anthal and Sahi, 2017).

All the species of Columbidae, Estrildidae and Passeridae family were found feeding on crop seeds and grains under granivorous guild. Pigeons and doves were foraging on seeds and grains fallen on ground at surrounding agricultural fields. Similar observations were made by Kumar and Sahu (2020). Herbivorous guild included 8 species feeding primarily on vegetation such as herbs, shrubs, shoots, roots, leaves of plants. The Eurasian coots were particularly seen foraging on macrophytes on floating water. The coots were observed feeding on ground and diving to get food in water. In frugivorous guild, parakeets were recorded feeding on fruits and buds of plants particularly guava, and similar observations were made by Clergeau and

Table 1. Feeding guilds of avifauna of Keshopur wetland

S. No.	Name of species	Scientific name	Resident status	IUCN status	Feeding guild
	Order-Accipitriformes, Family-Accipitridae				
1.	Black kite	*Milvus migrans* (Boddaert,1783)	R	LC	C
2.	Lesser spotted eagle	*Clanga pomarina* (Brehm, 1831)	R	LC	C
3.	Oriental honey buzzard	*Pernis ptilorhynchus* (Temminck, 1821)	R	LC	C
4.	Western marsh harrier	*Circus aeruginosus* (Linnaeus, 1758)	WM	LC	C
	Order-Anseriformes, Family-Anatidae				
5.	Bar-headed goose	*Anser indicus* (Latham, 1790)	WM	LC	H
6.	Common pochard	*Aythya ferina* (Linnaeus, 1758)	WM	VU	O
7.	Eurasian wigeon	*Mareca penelope* (Linnaeus, 1758)	WM	LC	H
8.	Ferruginous duck	*Aythya nyroca* (Güldenstädt, 1770)	WM	NT	O
9.	Gadwall	*Anas strepera* (Linnaeus, 1758)	WM	LC	H
10.	Greylag goose	*Anser anser* (Linnaeus, 1758)	WM	LC	H
11.	Indian spot billed duck	*Anas poecilorhyncha* (Forster, 1781)	R	LC	H
12.	Lesser whistling duck	*Dendrocygna javanica* (Horsefield, 1821)	SM	LC	O
13.	Mallard	*Anas platyrhynchos* (Linnaeus, 1758)	WM	LC	H
14.	Northern pintail	*Anas acuta* (Linnaeus, 1758)	WM	LC	H
15.	Northern shoveler	*Spatula clypeata* (Linnaeus, 1758)	WM	LC	O
	Order-Apodiformes, Family-Apodidae				
16.	Little swift	*Apus affinis* (Gray, 1830)	R	LC	I
	Order- Bucerotiformes, Family-Bucerotidae				
17.	Indian grey hornbill	*Ocyceros birostris* (Hume, 1873)	R	LC	O
	Order-Bucerotiformes, Family-Upupidae				
18.	Common hoopoe	*Upupa epops* (Linnaeus, 1758)	R	LC	O
	Order-Ciconiiformes, Family-Ciconiidae				
19.	Asian openbill	*Anastomus oscitans* (Boddaert, 1783)	WM	LC	C
20.	Painted stork	*Mycteria leucocephala* (Pennant, 1769)	R	NT	C
	Order-Charadriiformes, Family-Charadriidae				
21.	Little ringed plover	*Charadrius dubius* (Scopoli, 1786)	WM	LC	I
22.	Pacific golden plover	*Pluvialis fulva* (Gmelin, 1789)	WM	LC	NR
23.	Red-wattled lapwing	*Vanellus indicus* (Boddaert, 1783)	R	LC	MG
24.	White tailed lapwing	*Vanellus leucurus* (Lichtenstein, 1823)	WM	LC	C
25.	Yellow-wattled lapwing	*Vanellus malabaricus*(Boddaert, 1783)	R	LC	I
	Order-Charadriiformes, Family-Jacanidae				
26.	Pheasant tailed jacana	*Hydrophasianus chirurgus* (Scopoli, 1786)	R	LC	O
	Order-Charadriiformes, Family-Laridae				
27.	Brown headed gull	*Chroicocephalus brunnicephalus* (Jerdon, 1840)	WM	LC	NR
28.	Black headed gull	*Chroicocephalus ridibundus* (Linnaeus, 1766)	WM	LC	NR
29.	River tern	*Sterna aurantia* (Gray, 1831)	WM	NT	C
	Order- Charadriiformes, Family-Recurvirostridae				
30.	Black winged stilt	*Himantopus himantopus* (Linnaeus, 1758)	R	LC	I
	Order-Charadriiformes, Family-Scolopacidae				
31.	Common greenshank	*Tringa nebularia* (Gunnerus, 1767)	WM	LC	C
32.	Common sandpiper	*Actitis hypoleucos* (Linnaeus, 1758)	WM	LC	C
33.	Marsh sandpiper	*Tringa stagnatilis* (Bechstein, 1803)	WM	LC	C
34.	Spotted redshank	*Tringa erythropus* (Pallas, 1764)	WM	LC	C
	Order- Columbiformes, Family-Columbidae				
35.	Eurasian collared dove	*Streptopelia decaocto*(Frivaldszky, 1838)	R	LC	G
36.	Laughing dove	*Spilopelia senegalensis* (Linnaeus, 1766)	R	LC	G
37.	Oriental turtle dove	*Streptopelia orientalis* (Latham, 1790)	R	LC	G
38.	Red collared dove	*Streptopelia tranquebarica* (Hermann, 1804)	R	LC	G
39.	Rock pigeon	*Columba livia* (Gmelin, 1789)	R	LC	G
40.	Spotted dove	*Spilopelia chinensis* (Scopoli, 1786)	R	LC	G
41.	Yellow-footed green pigeon	*Treron phoenicoptera* (Latham, 1790)	R	LC	G
	Order-Coraciiformes, Family-Alcedinidae				
42.	Lesser pied kingfisher	*Ceryle rudis* (Linnaeus, 1758)	R	LC	C
43.	White-throated kingfisher	*Halcyon smyrnensis* (Linnaeus,1758)	R	LC	C

(contd.)

Table 1 (contd.)

	Order-Coraciiformes, Family-Coraciidae				
44.	Indian roller	*Coracias benghalensis* (Linnaeus, 1758)	R	LC	C
	Order-Coraciiformes, Family-Meropidae				
45.	Green bee eater	*Merops orientalis* (Latham, 1801)	R	LC	I
46.	Blue tailed bee eater	*Merops philippinus* (Linnaeus, 1766)	SM	LC	I
	Order-Cuculiformes, Family-Cuculidae				
47.	Asian koel	*Eudynamys scolopaceus* (Linnaeus, 1758)	R	LC	O
48.	Greater coucal	*Centropus sinensis* (Stephens, 1815)	R	LC	O
	Order-Falconiformes, Family-Falconidae				
49.	Peregrine falcon	*Falco peregrines* (Tunstall, 1771)	R	LC	C
	Order-Galliformes, Family-Phasianidae				
50.	Black francolin	*Francolinus francolinus* (Linnaeus, 1766)	R	LC	NR
	Order-Gruiformes, Family-Rallidae				
51.	White breasted waterhen	*Amaurornis phoenicurus* (Pennant, 1769)	R	LC	O
52.	Purple swamphen	*Porphyrio porphyrio* (Linnaeus, 1758)	R	LC	O
53.	Common moorhen	*Gallinula chloropus* (Linnaeus, 1758)	R	LC	O
54.	Eurasian coot	*Fulica atra* (Linnaeus, 1758)	WM	LC	H
	Order-Passeriformes, Family-Alaudidae				
55.	Ashy-crowned sparrow lark	*Eremopterix griseus* (Scopoli, 1786)	R	LC	NR
56.	Crested lark	*Galerida cristata* (Linnaeus, 1758)	R	LC	NR
	Order-Passeriformes, Family-Campephagidae				
57.	White-bellied minivet	*Pericrocotus erythropygius* (Jerdon, 1840)	R	LC	I
	Order- Passeriformes, Family-Cisticolodae				
58.	Yellow-bellied prinia	*Prinia flaviventris* (Delessert, 1840)	R	LC	I
	Order-Passeriformes, Family-Corvidae				
59.	Common raven	*Corvus corax* (Linnaeus, 1758)	R	LC	O
60.	House crow	*Corvus splendens* (Vieillot, 1817)	R	LC	O
61.	Indian jungle crow	*Corvus macrorhynchos* (Wagler, 1827)	R	LC	O
62.	Indian treepie	*Dendrocitta vagabunda* (Latham, 1790)	R	LC	I
	Order-Passeriformes, Family-Dicruridae				
63.	Ashy drongo	*Dicrurus leucophaeus*	R	LC	I
64.	Black drongo	*Dicrurus macrocercus* (Viellot, 1817)	R	LC	I
	Order-Passeriformes, Family-Estrildidae				
65.	Scaly breasted munia	*Lonchura punctulata* (Linnaeus, 1758)	R	LC	G
66.	Tricolour munia	*Lonchura Malacca* (Linnaeus, 1766)	R	LC	G
	Order- Passeriformes, Family-Laniidae				
67.	Long tailed shrike	*Lanius schach* (Linnaeus, 1758)	R	LC	I
	Order-Passeriformes, Family-Monarchidae				
68.	Asian paradise flycatcher	*Terpsiphone paradise* (Linnaeus, 1758)	SM	LC	I
	Order-Passeriformes, Family-Motacillidae				
69.	Western yellow wagtail	*Motacilla flava* (Linnaeus, 1758)	WM	LC	I
70.	Grey wagtail	*Motacilla cinerea* (Tunstall, 1771)	WM	LC	I
71.	Citrine wagtail	*Motacilla citreola* (Pallas, 1776)	WM	LC	I
72.	White browed wagtail	*Motacilla maderaspatensis* (Gmelin, 1789)	R	LC	I
73.	Paddy field pipit	*Anthus rufulus* (Vieillot, 1818)	R	LC	I
74.	Long billed pipit	*Anthus similis* (Jerdon, 1840)	WM	LC	I
	Order-Passeriformes, Family-Muscicapidae				
75.	Oriental magpie robin	*Copsychus saularis* (Linnaeus, 1758)	R	LC	I
76.	Indian black robin	*Copsychus fulicatus* (Linnaeus, 1766)	R	LC	I
77.	Bluethroat	*Luscinia svecica* (Linnaeus, 1758)	R	LC	I
	Order-Passeriformes, Family-Nectariniidae				
78.	Purple sunbird	*Cinnyris asiaticus* (Latham, 1790)	R	LC	N
	Order-Passeriformes, Family-Passeridae				
79.	House sparrow	*Passer domesticus* (Linnaeus, 1758)	R	LC	G
	Order-Passeriformes, Family-Ploceidae				
80.	Baya weaver	*Ploceus philippinus*(Linnaeus,1766)	R	LC	O
81.	Streaked weaver	*Ploceus manyar* (Horsfield, 1821)	R	LC	O
82.	Black breasted weaver	*Ploceus benghalensis* (Linnaeus, 1758)	R	LC	O
	Order-Passeriformes, Family-Pycnonotidae				
83.	Red vented bulbul	*Pycnonotus cafer* (Linnaeus, 1766)	R	LC	O
84.	White eared bulbul	*Pycnonotus leucotis* (Gould, 1836)	R	LC	O

(contd.)

Table 1 (contd.)

	Order- Passeriformes, Family-Rhipiduridae				
85.	White throated fantail	*Rhipidura albicollis* (Vieillot, 1818)	R	LC	I
	Order-Passeriformes, Family-Sturnidae				
86.	Asian pied starling	*Gracupica contra* (Linnaeus, 1758)	R	LC	O
87.	Bank myna	*Acridotheres ginginianus* (Latham, 1790)	R	LC	I
88.	Common myna	*Acridotheres tristis* (Linnaeus, 1766)	R	LC	O
	Order-Passeriformes, Family-Sylviidae				
89.	Common tailor bird	*Orthotomus sutorius* (Pennant, 1769)	R	LC	I
	Order-Passeriformes, Family-Timaliidae				
90.	Common babbler	*Argya caudata* (Dumont, 1823)	R	LC	O
91.	Jungle babbler	*Argya striata* (Lesson, 1831)	R	LC	O
	Order-Passeriformes, Family-Zosteropidae				
92.	Oriental white eye	*Zosterops palpebrosus* (Temminck, 1824)	R	LC	I
	Order-Pelecaniformes, Family-Ardeidae				
93.	Black crowned night heron	*Nycticorax nycticorax* (Linnaeus, 1758)	R	LC	C
94.	Cattle egret	*Bulbulcus ibis* (Linnaeus, 1758)	R	LC	C/I
95.	Eurasian bittern	*Botaurus stellaris* (Linnaeus, 1758)	R	LC	C
96.	Great egret	*Ardea alba* (Linnaeus, 1758)	R	LC	C
97.	Grey heron	*Ardea cinerea* (Linnaeus, 1758)	R	LC	C
98.	Indian pond heron	*Ardeola grayii* (Skyes, 1832)	R	LC	C
99.	Intermediate egret	*Ardea intermedia* (Wagler, 1827)	R	LC	C
100.	Little egret	*Egretta garzetta* (Linnaeus, 1766)	R	LC	C
101.	Purple heron	*Ardea purpurea* (Linnaeus, 1766)	R	LC	C
102.	Yellow bittern	*Ixobrychus sinensis* (Gmelin, 1789)	R	LC	C
	Order-Pelecaniformes, Family-Threskiornithidae				
103.	Glossy ibis	*Plegadis falcinellus* (Linnaeus, 1766)	R	LC	C
104.	Indian black ibis	*Pseudibis papillosa* (Temminck, 1824)	R	LC	C
105.	Black headed ibis	*Threskiornis melanocephalus* (Latham, 1790)	R	NT	C
	Order-Piciformes, Family-Capitonidae				
106.	Blue throated barbet	*Psilopogon asiaticus* (Latham, 1790)	R	LC	F
107.	Brown headed barbet	*Psilopogon zeylanicus* (Gmelin, 1788)	R	LC	F
108.	Coppersmith barbet	*Psilopogon haemacephalus* (Muller, 1776)	R	LC	F
	Order-Piciformes, Family-Picidae				
109.	Black rumped flameback	*Dinopium benghalense* (Linnaeus, 1758)	R	LC	NR
	Order-Podicipediformes, Family-Podicipedidae				
110.	Little grebe	*Tachybaptus ruficollis* (Pallas, 1764)	R	LC	C
111.	Black necked grebe	*Podiceps nigricollis* (Brehm, 1831)	WM	LC	C
	Order-Psittaciformes, Family-Psittaculidae				
112.	Slaty headed parakeet	*Psittacula himalayana* (Lesson,1832)	R	LC	F
113.	Plum headed parakeet	*Psittacula cyanocephala* (Linnaeus,1766)	R	LC	F
114.	Rose ringed parakeet	*Psittacula krameri* (Scopoli, 1769)	R	LC	F
115.	Alexandrine parakeet	*Psittacula eupatria* (Linnaeus, 1766)	R	NT	F
	Order-Suliformes, Family-Anhingidae				
116.	Oriental darter	*Anhinga melanogaster* (Pennant, 1769)	R	NT	NR
	Order-Suliformes, Family-Phalacrocoracidae				
117.	Indian cormorant	*Phalacrocorax fuscicollis* (Stephens, 1826)	R	LC	C
118.	Great cormorant	*Phalacrocorax carbo* (Linnaeus, 1758)	R	LC	C
119.	Little cormorant	*Microcarbo niger* (Vieillot, 1818)	R	LC	C
	Order-Strigiformes, Family-Strigidae				
120.	Indian eagle owl	*Bubo bengalensis* (Franklin, 1831)	R	LC	NR
121.	Spotted owlet	*Athene brama* (Temminck, 1821)	R	LC	C

Resident status: R- Resident; WM- Winter migratory; SM- Summer migratory, IUCN status: V- Vulnerable; NT- Near threatened; LC- Least concern, Feeding guild: C- Carnivorous; O- Omnivorous; I- Insectivorous; H- Herbivorous; G-Granivorous; F- Frugivorous; N- Nectarivorous; MG- Multiple Guild; NR- Not recorded

Vergnes (2011). Purple sunbird (*Cinnyris asiaticus*) feeds on nectar of flowers. Nectarivorous bird can also feed on fruit juice (Ghadirian et al., 2008). Cattle egret and red-wattled lapwing, members of carnivorous guild were observed to feed on insects also, hence placed in multiple guilds. The foraging activities of nine species could not be observed as these were mostly roosting. Feeding is an important activity in the life of the bird which is indispensible for their survival (Panda et al., 2021). Feeding guilds of birds provide insight into the

ecology of species and are particularly useful in studies that assess specific ecological drivers of community change. Species belonging to the same guild utilize the same kind of resource in a similar manner. Guild categorization among birds emphasizes upon functional component of community in an ecosystem. Keshopur wetland is biologically very productive and provides feeding grounds for a diverse range of resident and migratory birds.

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ECOLOGY OF *OTINOTUS ONERATUS* WALKER (HEMIPTERA: MEMBRACIDAE) ON ITS HOST PLANT *TAMARINDUS INDICA*

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ABSTRACT

This study is on the ecology and population dynamics of the membracid *Otinotus oneratus* Walker on its host plant *Tamarindus indica* L. The daily activity of *O. oneratus* when analysed revealed that it was active during 11:00 and 16:00 hr, and on extremely warmer days. Adults preferred woody internodal parts, while the early nymphal stages fed on the axil of the leaves, and the later nymphs fed on the internodal parts along with the adults. The resting posture of the adults were found to be in position with its head directed upwards on its host plant. The mean number of nymphs resting in a posture facing towards and away from node was 79.7 and 17.1 respectively; with adults, for males and females it was 3.0 and 4.6 upwards facing, and for downwards it was 2.4 and 3.6, respectively. The individuals were sluggish, and when disturbed, moved in a spiral manner around the twig, and stopped after moving some distance. Mating occurred for 5 min, followed by a female preoviposition period of 3 days. Eggs were elongate and white and laid in 'V' shaped manner, arranged in two rows with 12-19 eggs in each row. Parental care was exhibited with the females guarding 71.4 to 83.3% of egg masses. The incubation period varied from 8-12 days. Hatching occurred during the morning hours, and the nymphs were slow moving, showing a strong myrmecophilous mutualism, regularly attended by the large black ant *Camponotus compressus* F. (56.5- 92.9%). Predation by *Gonatocerus brevifuniculatus* Subba Rao and *Mirufens afrangiata* Viggiani and Hayat of eggs wqas observed. *O. oneratus* nymphs mimicked the thorns of the host plant, but they were brightly coloured (in contrast to the twigs). The occurrence of nymphs and adults reached a peak in December and January. The present study also assessed the population dynamics in relation to weather factors.

Key words: *Otinotus oneratus*, *Tamarindus indica*, bioecology, behavioure, parental care, ant attendance, predation, mimicry, colouration, population dynamics, weather factors

The family Membracidae comprises small to medium sized phytophagous insects popularly known as 'cow bugs', 'horn bugs', and 'tree hoppers'. The existence of intraspecific variations among the membracids was established through the contributions of Ananthasubramanian and Ananthakrishnan (1975a) on Indian species. In India, these insects are represented by three subfamilies, viz., Oxyrhachinae, Centrotinae and Darthulinae and have not received an adequate attention except for the habitual aspects (Behura and Panda, 1951), behavioural and bioecological studies (Ananthasubramanian and Ananthakrishnan, 1975a,b), and biosystematics (Ananthasubramanian, 1987). Misra et al. (2003) and Sharma and Pati (2011) recorded the presence of *Oxyrhachis tarandus* F. on mulberry tree and *Withania somnifera* (L.) Dunal, respectively. Nettimi and Iyer (2015) and Radha and Susheela (2016) reported on the myrmecophilous mutualism between treehoppers and ants. Recently Rajkumar et al. (2021) reported on the behavioural aspects of *Leptocentrus taurus* Fabricius, *O. oneratus* and *O. taranda* on their host plants. Remarkably, the population dynamics of membracids have been investigated in relation to weather parameters. Funkhouser (1917) studied ecological factors like temperature and humidity on the membracid species, while Ananthasubramanian and Ananthakrishnan (1975b) and Devarajan (1984) made preliminary studies on the population dynamics in relation to weather factors of common membracids, viz., *O. oneratus* and *Oxyrhachis rufescens* Walker, respectively. Mitchell and Newsome (1984) reported that the seasonal changes in temperature influenced the nymphal duration, egg incubation period and oviposition behaviour of the three cornered alfalfa hopper *Spissistilus festinus* Say. Nixon and Thompson (1987) stated the effect of temperature on hatching and nymphal development of membracids. Rajkumar et al. (2001, 2011a,b,c) evaluated the population dynamics of

three membracids, viz., *L. taurus*, *O. oneratus*, and *O. taranda*. The association between the membracid and the host plant is so characteristic that the knowledge of the one is sufficient to recognise the other. Though this statement holds well for several species of Indian membracids, the species in the present study is polyphagous. Consequently, the present research has documented its report on the behavioural, bioecological and population dynamics aspects of *O. oneratus* on its host plant *T. indica*.

MATERIALS AND METHODS

The membracid, *O. oneratus* was studied by regular field observations, and collection was done by hand picking every fortnight by choosing ten twigs of *T. indica* in and around Binny Quarters at Pattalam near Perambur Barracks road, Chennai, Tamil Nadu, India (13.1180566°N, 80.2404789°E) for a period of eight months from September 2018 to April 2019. The glass tube enclosure method was used to study the bioecological and behavioural aspects, viz., feeding, resting, locomotion, mating, oviposition, parental care, and the honeydew secretion by nymphs when ants were introduced, besides mimicry and colouration. Live branches of its host plant containing adults were enclosed in a specially designed glass tube open at both ends. One end of the tube was closed by a cork, made up of two halves, which was grooved in the middle in such a way that the nymphs will not escape. The host plant twig was inserted through the tube so as to enclose the eggs, nymphs and the adults. The other end of the glass tube was plugged with cotton wool which prevented escape from the tube. Egg clusters were collected and reared in test tube enclosure to study the incubation period, hatching, and the duration of immature stages of this insect. Laboratory cultures were maintained for supplementing and confirming the field observations. Fresh twigs of its host plant containing egg clusters or nymphs of different stages were cut at the basal end and dipped in Knop's solution kept in a conical flask covered with a rubber cork. The twigs were enclosed in a mesh chamber kept over the conical flask. The twigs were replaced by fresh ones, once in four days. Nylon nets with medium sized meshes were tied on to the host plant for close observation of predation by natural enemies. The population dynamics of this bug was assessed in relation to the weather parameters, viz., temperature, relative humidity, rainfall, and wind speed, and their data were obtained from Meteorological Research Station, Nungambakkam, Chennai, Tamil Nadu, India. Statistical analysis was performed using SPSS software (SPSS, 2010) to work out the correlation coefficients of incidence of nymphs and adults of *O. oneratus* with weather factors.

RESULTS AND DISCUSSION

Field observations in the present study documented that *O. oneratus* was found on the young tender twigs of *T. indica*. The nymphs led a gregarious life during their early stages, while the later nymphal and adults were solitary. The daily activity observed with reference to their behavioural aspects was found to be more active during 11:00 and 16:00 hr, and on extremely warmer days. This diurnal rhythm may be due to the fact that many predators of this bug are less in numbers during the heat of the day, however, this may be only an assumption and it requires more intensive studies to confirm or disprove it. Feeding was observed at almost any hour of the day, however, the most favoured time was in the mid afternoon. Adults preferred woody internodal parts, while the early nymphal stages fed on the axil of the leaves, and the later nymphs fed on the internodal parts along with the adults. The feeding behaviour of *O. oneratus* is a leisurely one (Funkhouser, 1951). During this study, the nymphs and adults remained in one spot for a long time, and showed little disposition to seek new feeding places. Most adults rested on horizontal branches with their head directed upward, and away from the main trunk. The mean number of males and females facing upward and downward was 3.0 and 4.6, and 2.4 and 3.6, respectively. The mean number of nymphs facing towards and away from node was 79.7 and 17.1, respectively (Fig. 1). Conversely, the downward posture may be an adaptation to jump down to the ground at the sign of danger as reported by Devarajan (1984) in *O. rufescens* adults whose head faced downwards.

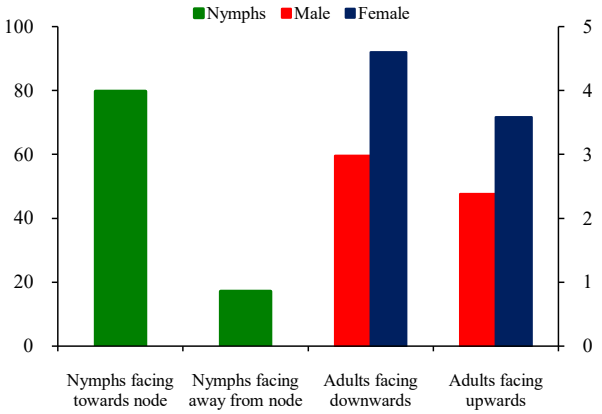


Fig. 1. Resting posture of *O. oneratus*

Oxyrhachis oneratus movement was sluggish, and when disturbed, moved in a spiral manner around the twig of the host plant, and stopped their movement after moving to some distance. Adult males were more easily disturbed than the females and they exhibited a tendency to take to their wings on continued disturbance. Nymphs were incapable of flight when disturbed, and crawled around the twig in a spiral fashion, and settled down on a node far away from the point of disturbance as reported by Funkhouser (1951). A single female was surrounded by four to seven males, and for unknown reasons, all but one moved away in the present study. The pair remained for several hours in copula with their heads facing opposite directions. Mating occurred for 5 min, and thereafter, the male disengaged and moved away, and the female passed into the preoviposition period, and invariably oviposited on the young twigs of the host plant. Observation made on the mating behaviour of *Platycotis vittata* F. by Wood et al. (1984) showed that females mate up to five times, and the present study also revealed similar sequences.

Preoviposition period of 5-10 days was observed after mating (Funkhouser, 1951), while in the present study, it occurred for 3 days. Females possessed strong and powerful ovipositors, and searched for a suitable ovipositing site on the host plant, before making the puncture. In the act of oviposition, the ovipositor was held at right angles to her abdomen, and numerous eggs were laid in 'V' shaped manner, arranged in two rows wherein each row converged at one end and diverged at the other, and each row contained 12-19 eggs. The eggs were shiny white, elongate, with a slight curvature, while its basal part was round and its tip appeared club-like as in *L. leucaspis* Walker (Ananthasubramanian and Ananthakrishnan, 1975b). Membracids exhibit maternal care to the nymphs (Urquizo et al., 2020). In the present study, of *O. oneratus* guarded 71.4 to 83.3% of egg masses (Fig. 2), and in case a female was dislocated, another female took over the duty of guarding the eggs. Further, they showed aggressive behaviour to the enemies, by repelling them, thereby increasing the survival value of the eggs.

The incubation period was for a minimum of eight days and a maximum of 12 days, and extended to more than nine days in October and November (Fig. 3). This difference in the incubation period appear might be due to the effects of temperature, moisture, and humidity as extreme temperature and low humidity may retard embryonic development (Ananthasubramanian and Ananthakrishnan, 1975a,b). Hatching occurs during the

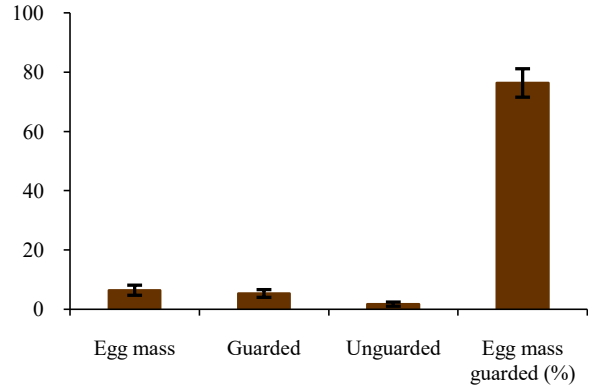


Fig. 2. Parental care of *O. oneratus*

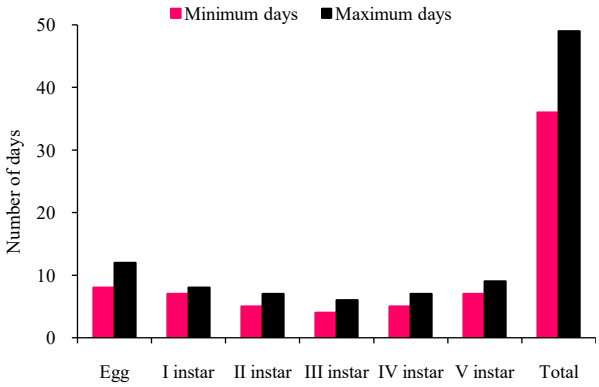


Fig. 3. Incubation period of *O. oneratus*

morning, and the whole process of hatching takes about 15 to 30 min, prior to which, the eggs increase in size, turns yellow, and the duration of egg stage differs, with a minimum of 5 days, and to a maximum of 16 days (Ananthasubramanian and Ananthakrishnan, 1975b). The same was observed in the present study too. During hatching, the hatching membrane which enclosed the embryo ruptured, the egg cap was forced upwards, and the head of the nymph projected out. The nymphs then pulled the terminal part of their abdomen out of the shell and rested for a while. Nymphal stages of membracids are caressed by ants for their anal secretion which are consumed by ants, and they in turn afford protection against predators and natural enemies (Nettimi and Iyer, 2015; Radha and Susheela, 2016). In the present study, *O. oneratus* nymphs were attended by the large black ant *C. compressus*, and the ant attendance was between 56.5 and 92.9% (Fig. 4). Similar observations were made by Sharma and Sundarraj (2011) on *Leptocentrus longispinus* Distant, *O. oneratus* and *O. tarandus*; and by Seni (2021) on *O. oneratus* and *L. taurus* which also demonstrated the value of ant attendance in increasing the survival value of nymphs. The nymphs behaviour ensured easy honey dew collection by ants, thereby

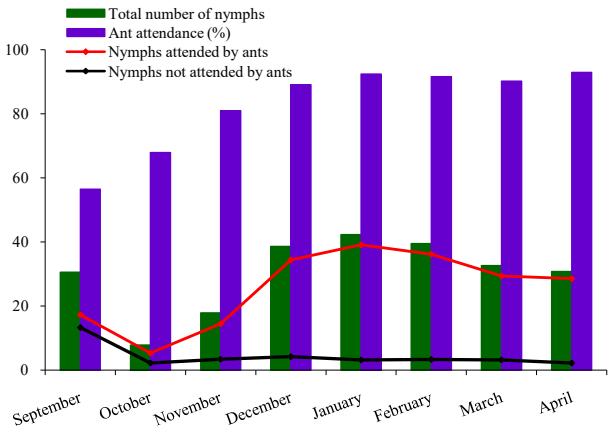


Fig. 4. Ant attendance to *O. oneratus*

making trophobiosis a success as reported by Gjonov and Lapeva-Gjonova (2013).

In the present study, *G. brevifuniculatus* and *M. afrangiata* attacked the eggs of *O. oneratus*. No nymphal parasitoids was observed. Similar predation was reported by Ananthasubramanian and Ananthakrishnan (1975b) on membracid eggs by chalcidoid hymenopterans. Ananthasubramnian and Ananthakrishnan (1975a) proved the value of mimetic behaviour and cryptic colouration in the survival rate of membracids wherein they reported the mimetic nature of membracids body form and colour patterns that blend remarkably with the bark, flowers, buds, stipules, spines, and fruits of their host plants. In the present study, *O. oneratus* nymphs mimicked the thorns of the host plant, but with regard to their colouration, they were brightly coloured in contrast to the colour of the twigs on which they inhabited, and therefore were easily attacked by predators.

The population of *O. oneratus* gradually increased from December 2018 to April 2019, and its density showed the highest peak in the months of December, January and February. Decrease in their population was observed in the months of October and November (Fig. 5). Among the nymphs, the population of I instar was more when compared to other nymphal instars, and V instar was the least (Fig. 6). The adult population showed more number of females when compared to males. The average male: female sex ratio was 1:4.1, and was maximum in October (1: 5.4) and February (1:5.7) (Fig. 7). Student 't' test performed between the nymphs and adults was found to have a high level of significance between them, and the calculated 't' test value between males and females was found to be greater than the table value at 95% level of significance which indicated a significant difference between them

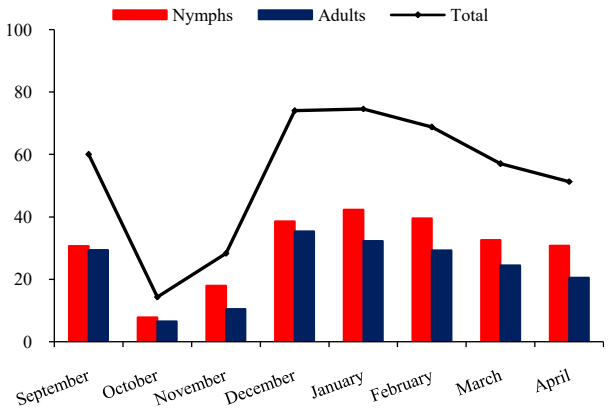


Fig. 5. Population dynamics of *O. oneratus* -adults

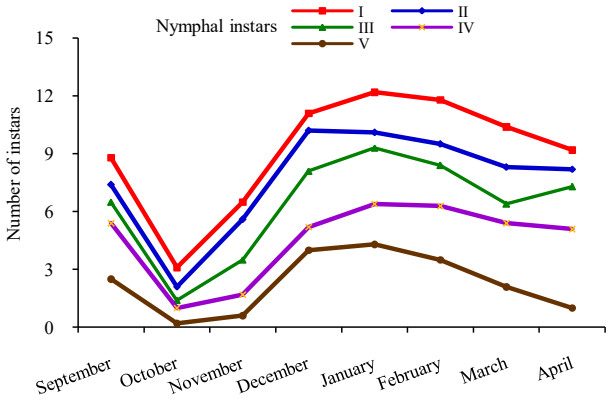


Fig. 6. Population dynamcis of *O. oneratus*- nymphs

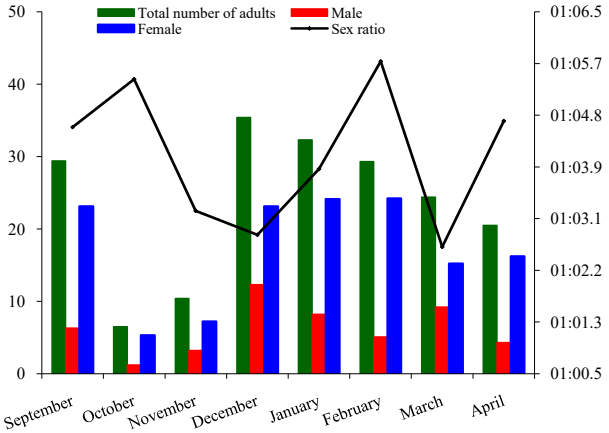


Fig. 7. Sex ratio of *O. oneratus*

(Table 1). The weather parameters, viz., temperature, relative humidity, rainfall, and wind speed played a vital role in changing the population structure (Fig. 8), and the fluctuations were statistically correlated (Table 2).

The present study brought to light that the weather parameters played a significant role on the population fluctuation of *O. oneratus* and their peak period based on the seasonal periods. Temperature played a vital role

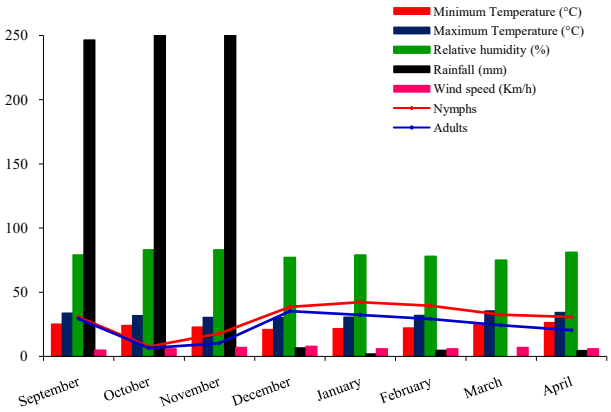


Fig. 8. Effect of weather parameters on *O. oneratus*

Table 1. Student 't' test analysis between nymphal and adult populations of *O. oneratus*

Nymphal and adult population	Mean± S.D.	't' value
Total with I instar	-20.88± 8.70	-6.785*
Total with II instar	-22.34± 9.06	-6.971*
Total with III instar	-23.65± 9.10	-7.351*
Total with IV instar	-25.45± 9.78	-7.363*
Total with V instar	1.46± 10.33	-7.594*
I with II instar	2.78± 0.61	6.815*
I with III instar	4.58± 0.78	10.188*
I with IV instar	6.86± 1.31	9.852*
I with V instar	1.31± 1.85	10.483*
II with III instar	3.11± 0.61	6.081*
II with IV instar	5.40± 1.19	7.414*
II with V instar	1.80± 1.59	9.605*
III with IV instar	4.09± 0.91	5.612*
III with V instar	2.29± 1.52	7.602*
IV with V instar	2.29± 1.18	5.471*
Total with male	-47.31± 19.07	-7.02*
Total with female	-36.24± 14.60	-7.02*
Female with male	-11.08± 5.92	-5.29*
Total with nymphs	-23.53± 10.41	-6.40*
Total with adults	-30.01± 11.73	-7.23*
Nymphs with adults	-6.49± 3.97	-4.62*

on the nymphal and adult populations. Moderate rainfall did not have any substantial influence, however, heavy rainfall did reflect on its population density in October and November. The population reached the peak in December and January due to congenial condition involving moderate temperature, relative humidity, rainfall and wind speed. Ali et al. (2008) reported that the population density of *O. tarandus* was associated with temperature, relative humidity, rainfall, and vapour pressure. Lopes (1995) reported that the abundance of membracids were high from the months of October to February, while from March to September, they showed a decrease in their numbers. Rajkumar et al. (2001) reported that the population density of *O. taranda*

Table 2. Statistical inferences between population of *O. oneratus* and weather parameters

Weather parameters	Correlation coefficient	Regression equation	't' value
Minimum temperature (°C)	-0.394	Y=25.223-7.06E-02X	-1.046
Maximum temperature (°C)	-0.030	Y=32.471-5.66E-03X	-0.073
Relative humidity (%)	-714	Y=330.998-9.076X	-2.281
Rainfall (mm)	-776**	Y=84.331-0.211X	-3.013
Wind speed (Km/h)	0.084	Y=6.200+7.422E-03X	0.207

*Significant at p=0.01; **Significant at p=0.05%

attained its peak in September, declined in October due to heavy rainfall, and was then stable from December to February, and thereafter in March, April and May, decreased due to increase in temperature. It was shown that the population fluctuation of both the nymphs and adults had a positive correlation to temperature only to a certain extent, because at very high temperature, the population was affected and the population remained quite static. The population of *L. taurus* (Rajkumar et al., 2011a), *O. oneratus* (Rajkumar et al., 2011b) and *O. taranda* (Rajkumar et al., 2011c) reported highest peaks in the months of October, November and December due to rainfall, and its decline in May due to high temperature, besides less rainfall and relative humidity. Bijewar et al. (2018) and Brahman et al. (2018) also reported that the population of *O. oneratus* was high in September and October. Henceforth, the precise role of various abiotic and biotic factors on the population fluctuation of membracids are to be determined by further studies.

Although the present findings has brought to light the salient ecological and behavioural aspects of *O. oneratus*, future line of investigations are needed, and studies on membracids exposed to different species of plants may show more ecological and biological aspects when ventured in different geographical regions of host diversity.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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PERFORMANCE OF AROMATIC RICE VARIETIES AGAINST YELLOW STEM BORER *SCIRPOPHAGA INCERTULAS*

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ABSTRACT

A field experiment was carried out at the Rice Research Farm of Birsa Agricultural University, Ranchi, Jharkhand during kharif, 2018 and 2019 to assess the performance of some aromatic rice varieties against the yellow stem borer (YSB) *Scirpophaga incertulas* (Pyralidae: Lepidoptera) in Jharkhand. The damage caused was measured as deadheart- DH (30 and 45 DAT) and white earhead- WEH (70 and 90 DAT) at the vegetative and reproductive stages, respectively. At vegetative stage seven varieties scored 1 (PS-4, Pusa1176, PS-3, BVS-1, BR-9, BR-10, Assam culture) with 4.62 to 9.53% DH while at reproductive stage only two varieties (BR-10 and Assam Culture) scored 1 with 4.56 and 4.95% WEH. Yield potential of BR-9 (38.50 q/ ha) was significantly superior over other aromatic varieties except BR-10 and followed by Birsamati (34.30 q/ ha) and BVS-1 (33.25 q/ ha).

Key words: *Scirpophaga incertulas*, aromatic rice, deadheart, white earhead, varietal resistance, incidence, grain yield

Rice crop is subjected to attack by > 100 insect pests, and in Jharkhand among these pests, the yellow stem borer, hispa, green leaf hopper, leaf folder, gundhi bug and case worm account for 20-35% yield loss (Krishnaiah et al., 2008). In a particular area extent of damage by insect pest in non-aromatic rice is more which may be due to aromatic nature (Singh et al., 2010). The yellow stem borer *Scirpophaga incertulas* Walker (Pyralidae: Lepidoptera) is a monophagous pest and solely causes 2-20% damage (Satpathi et al., 2012). The management of larvae which cause damage is slightly difficult because it remains concealed inside the stem (Abro et al., 2013). Use of pest resistance and tolerant crop varieties is of immense value for raising the crop with no use or minimum use of insecticides, especially for farmers of state of Jharkhand who have poor socio-economic background. This study evaluates some aromatic rice varieties for their resistance to *S. incertulas.*

MATERIALS AND METHODS

A field experiment was conducted during kharif 2018 and 2019 at the Rice Research Farm of Birsa Agricultural University, Kanke, Ranchi (23°17' N82° 19'E, 625 masl). Sixteen varieties including three checks (aromatic susceptible check, non-aromatic susceptible check and non-aromatic resistance check) were sown in nursery on 5th July and 21 days old seedling transplanted to main field on 26th July. All the locally recommended package of practices except insecticide application were adopted. Sixteen varieties were arranged in randomized block design in three replications. The damage caused by the yellow stem borer *Scirophaga incertulas* in terms of deadheart- DH (30 and 45 DAT) and white earhead- WEH (70 and 90 DAT) was recorded by selecting five hills randomly in each replication. The % damage was calculated and converted to D value (Heinrich et al., 1985). After winnowing, grain yield was calculated and converted into q/ ha. Based on the damage grading of varieties was done following IRRI standard evaluation system.

RESULTS AND DISCUSSION

Pooled data on deadheart (DH) and white earhead (WEH) and yield are presented in Table 1. Among aromatic test varieties the DH values at prereproductive stage varied from 4.62 to 15.87% with minimum incidence 4.62% DH in BR-9 which was at par with Assam Culture (5.40% DH) and followed by BR-10 with 6.43% DH. All the varieties found superior over both the susceptible check variety. Among these test varieties, seven varieties scored 1 (PS-4, Pusa1176, PS-3, BVS-1, BR-9, BR-10, Assam culture) with 4.62 to 9.53 % DH, six varieties scored 3 with 10.89 to15.87 % DH. Aromatic susceptible check variety Pusa Basmati-1 scored 3 with 18.31 % DH, while non-aromatic susceptible check variety TN-1 scored 5 with

Table 1. Incidence of *S. incertulas* in aromatic rice varieties

Variety	Rice varieties	DH%			WEH%			Yield (q/ ha)
		DH%	D	Score	WEH %	D	Score	
V1	PS4	8.24 (16.64)	42.15	1	10.44 (18.84)	62.14	5	29.15
V2	Pusa1176	9.53 (17.97)	48.75	1	7.89 (16.30)	46.96	3	28.58
V3	PS-3	7.53 (15.92)	38.52	1	7.33 (15.70)	43.63	3	31.65
V4	PS-5	11.13 (19.47)	56.93	3	8.56 (16.99)	50.95	3	30.11
V5	Birsamati	10.89 (19.25)	55.70	3	9.65 (18.07)	57.44	3	34.30
V6	BVS-1	8.87 (17.32)	45.37	1	8.26 (16.70)	49.17	3	33.25
V7	BR-9	4.62 (12.39)	23.63	1	6.24 (14.46)	37.14	3	38.50
V8	BR-10	6.43 (14.68)	32.89	1	4.56 (12.28)	27.14	1	35.90
V9	Katarani	13.09 (21.20)	66.96	3	13.93 (21.90)	82.92	5	22.25
V10	BadshahBhog	10.96 (19.32)	56.06	3	13.09 (21.20)	77.92	5	26.33
V11	Assam Culture	5.40 (13.42)	27.62	1	4.95 (12.81)	29.46	1	29.55
V12	R. Kasturi	14.73 (22.55)	75.35	3	13.83 (21.82)	82.32	5	21.67
V13	R. Subhasani	15.87 (23.46)	81.18	3	13.01 (21.12)	77.44	5	24.35
V14	Pusa Basmati-1(SC)	18.31 (25.32)		3	15.83 (23.43)		7	20.17
V15	Suraksha (RC): Nonaromatic	3.44 (10.67)		1	3.31 (10.45)		1	32.60
V16	TN-1 (SC): Nonaromatic	20.79 (27.11)		5	17.76 (24.92)		7	18.17
SE m(±)		(0.43)			(0.54)			1.37
CD(P=0.05)		(1.24)			(1.32)			3.98
CV (%)		(4.10)			(4.50)			8.34

Figures in parentheses angular transformed values; DH: Deadheart, WEH: white earhead DAT: Days after transplanting

20.79 % DH. The converted D value for test varieties varies from 23.63 to 81.18 %. The descending order of resistance against DH % based on the basis of D value was BR-9(23.63) > Assam Culture (27.62) >BR-10 (32.89) > PS-3 (38.52) > PS-4(42.15) > BVS-1 (45.37) > Pusa 1176 (48.75) > Birsamati (55.70) > Badshah Bhog (56.06) >PS-5 (56.93) > Katarani (66.96) > R. Kasturi (75.35) > R. Subhasani (81.18).

At post reproductive stage incidence of WEH was 4. 56 to 13.93 % among test varieties with two varieties viz., BR-10 and Assam Culture scored 1 with 4.56 and 4.95 % WEH, respectively. Six varieties namely Pusa1176, PS-3,PS-5,Birsamati, BVS-1 and BR-9 scored 3 with 6.24 to 9.65 per WEH, and five varieties viz., PS-4, Katrani, Badshah Bhog, R. Kasturi and R. Sbhasani scored 5 with 10.44 to 13.93 % WEH. All the varieties were significantly superior over susceptible check Pusa Basmati-1 which recorded score 7. The converted D value for test varieties varies from 27.14 to 82.32 in the following descending order: BR-10(27.14) > Assam Culture (29.46) > BR-9 (37.14) > PS-3 (43.63) > Pusa1176 (46.96) BVS-1 (49.17) > PS-5 (50.95) > Birsamati (57.44) > PS-4 (62.14) > R.Subhasani (77.44), Badshah Bhog (77.92) > R. Kasturi (82.32) > Katarani (82.92).

Singh and Shukla (2007) found that among 86 rice accessions 43 were promising against stem borer whereas 9 were resistance. Khan et al. (2010) reported minimum white earheads (2.35%) in IRRI-6 followed by DR-83 (4.45 %) and KSK-282 (4.97%). Rajaduari and Kumar (2017) reported that the entries Gontra Bidhan 3, XR 99986 and NDR-97 had high level of resistance at both vegetative and reproductive stage. Samrmitha et al. (2021) evaluated 48 rice accessions against yellow stem borer in which 5 accessions recorded nil deadheart and white earhead. Nyaupane (2022) evaluated rice genotypes against yellow stem borer through sex pheromone trap and found that adoption of rice variety Sarju 52 could safeguard. Among all the test varieties highest yield was recorded in BR-9 (38.50q/ha) which was statistically at par with BR 10 and significantly superior. Maravi et al. (2019) reported that the aromatic rice variety PS-5 produced the highest grain yield (39.1q/ ha) over PS-4 (34.6q/ ha) and PS-3 (29.2q/ ha). It might be due to the differences in the varietal vigour, genetic differences and yield potential among the varieties. Similar type of results was also found by Sridhar et al. (2011) and Khatoon et al (2018).

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AUTHOR CONTRIBUTION STATEMENT

Sudeepa Kumari Jha, Pradeep Kumar Singh and Rabindra Prasad designed and conducted research. Sudeepa Kumari Jha and Md Monobrullah wrote manuscript.

CONFLICT OF INTEREST

No conflict of interest

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IMMATURE NUT FALL OF COCONUT AND INCIDENCE OF RUGOSE SPIRALING WHITEFLY *ALEURODICUS RUGIOPERCULATUS* MARTIN

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ABSTRACT

Studies on the immature nut fall in *Aleurodicus rugioperculatus* Martin infested coconut palms with low (<10 spirals/ leaflet), medium (10-20 spirals/ leaflet) and high (> 20 spirals/ leaflet) incidence in Dr YSRHU- Godavari Ganga hybrid and local East Coast Tall (ECT) variety were undertaken at the Dr YSRHU- Horticultural Research Station (HRS), Ambajipeta and Kalavalapalli plantations. The overall dropped nuts (%) was 4.84, 27.48 and 35.32% at Ambajipeta, while it was 5.50, 28.11 and 36.01% in Godavari Ganga hybrid palms with low, medium and high incidence during 2020-21 at Kalavalapalli. The % of dropped nuts in ECT variety with low incidence, respectively was representing the general dropping with 4.06 and 4.68% at Ambajipeta and Kalavalapalli, respectively during 2020-21. In case of ECT palms with medium and high incidence, the nut dropping was 22.33 and 28.51% (Ambajipeta), and 23.49 and 30.58% (Kalavalapalli).

Key words: *Aleurodicus rugioperculatus,* coconut, nut fall, incidence, Dr YSRHU- Godavari Ganga hybrid, East Coast Tall, low, medium, high, Ambajipeta, Kalavalapalli

Coconut (*Cocos nucifera* L.) commonly known as "tree of heaven", "tree of abundance", "tree of life" and "kalpavriksha" is devastated by incidence of several pests and diseases that not only deteriorate the quality of nuts but also reduced the vigour and yield. (Chowdappa et al*.,* 2018; Neeraja et al*.,* 2020). In the recent era, coconut palms are damaged with invasion of many new pests, predominantly whiteflies. Invasion of rugose spiraling whitefly (RSW) *Aleurodicus rugioperculatus* Martin has been reported from India in Tamil Nadu (Sundararaj and Selvaraj, 2017) and Andhra Pradesh (Chalapathi Rao et al*.,* 2018). This exotic and invasive pest feeds and reproduces prolifically on adaxial surface of coconut palm leaves (Sundararaj and Selvaraj, 2017; Srinivasan et al*.,* 2017) which leads to honey dew secretion facilitating the growth of sooty mould on leaves, which further degrades photosynthetic activity and vigour, resulting yield loss in coconut palms. Earlier, studies of Voegele (1989) estimated 50-70% loss due to the coconut hispid beetle *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae). Perera (1993) reported heavy occurrence of pests causing over 40% damage to the coconut leaves during an outbreak of *Opisina arenosella* Walker; its severe incidence caused nut yield reduction by 45.4% (Chandrika et al*.,* 2010). Rajapakse et al. (2010) reported 5% immature nut fall in coconut due to incidence of mealy bugs (*Dysmicocus* spp.), scale insects (*Aspidiotus destructor*), mites (*Dolichotetranychus* spp.), *Cyclodes omma* larvae and weevil (*Meridolus* spp.). However, literature on the dropped nuts in coconut palms due to *A. rugioperculatus* is very meagre. Nut yield reduction must be quantified so as to plan for IPM in coconut palms, and hence the present study to estimate the dropped nuts in relation to *A. rugioperculatus* incidence at different intensities.

MATERIALS AND METHODS

Studies on dropped nuts (%) in *A. rugioperculatus* infested coconut palms of Dr YSRHU- Godavari Ganga hybrid (35 and 13 years) and East Coast Tall (ECT) (45 and 25 years) with low (<10 spirals/ leaflet), medium (10- 20 spirals/ leaflet) and high (> 20 spirals/ leaflet) incidence were quantified at the Dr YSRHU- HRS, Ambajipeta (16°59'38"N, 81°95'36"E) and Kalavalapalli plantations (16°94'82"N, 81°63'98" E). Fifteen palms carrying developing nuts after fertilization i.e., three months and above from each cultivar were identified and marked by using simple random sampling technique (Rajapakse et al*.,* 2010). The data was recorded on annual leaf production, number of bunches, buttons, dropped nuts and harvested nuts/ palm at monthly intervals during 2020-21.

RESULTS AND DISCUSSION

The overall mean of dropped nuts was 7.80 (4.84%) in Dr YSRHU- Godavari Ganga hybrid with low *A. rugioperculatus* incidence, whereas comparatively more nut fall of 43.60 nuts (27.48%) and 55.60 nuts (35.32%) was observed with medium and high incidence during 2020-21 at the Dr YSRHU- H.R.S, Ambajipeta, respectively; while, in Dr YSRHU- Godavari Ganga hybrid palms, more nut dropping was observed (November 2020 to March 2021) comparatively with 5.20 (3.23%), 29.60 (18.74%) and 40.00 nuts (25.40%) than during its absence (April to October 2021) with 2.60 (1.61%), 13.80 (8.73%) and 15.60 nuts (9.92%) with low, medium and high incidence at the Dr YSRHU-HRS, Ambajipeta, respectively. The overall mean of dropped nuts was 8.80 nuts (5.50%) in Dr YSRHU-Godavari Ganga hybrid when the incidence was low; whereas comparatively higher nut fall of 43.20 nuts (28.11%) and 57.00 nuts (36.01%) was observed with medium and high incidence at Kalavalapalli. Similar observations were made in the Kalavalapalli plantations too. However, there was a conspicuous increase in nut dropping with incidence (November 2020 to March 2021) -about 3.49% (5.60 nuts), 17.46% (26.80 nuts) and 25.05% (39.60 nuts) as compared to 2.00% (3.20 nuts), 10.66% (16.40 nuts) and 10.97% (17.40 nuts) during April to October 2021.

The dropped nuts were at 4.80 nuts (4.06%) in East Coast Tall (ECT) with low incidence, but increased to 28.20 (22.33%) and 36.20 nuts (28.51%) with medium and high incidence at the Dr YSRHU- HRS, Ambajipeta. In East Coast Tall (ECT) palms, more nut dropping was observed at 3.40 (2.88%), 19.00 (15.04%) and 24.00 nuts (18.88%) (November 2020 to March 2021), but during its absence (April to October 2021) it reduced to 1.18 to 9.60% at the Dr YSRHU- H R S, Ambajipeta, respectively. It amounted to 4.68 nuts (4.40%) in East Coast Tall (ECT) with low incidence, reaching to 23.49 nuts (19.80%) and 26.40 nuts (22.14%) with medium and high incidence. at Kalavalapalli; similar observations were made at the Kalavalapalli plantations too; however, there was a conspicuous increase in nut dropping during November 2020 to March 2021 reaching 3.44 (4.40 nuts), 16.98 (19.80 nuts) and 22.14% (26.40 nuts) compared to 1.24 (1.60 nuts), 6.50 (7.60 nuts) and 8.43% (10.00 nuts) noticed during April to October 2021 (Table 1).

Thus, the nut dropping was observed to be more in case of palms with high incidence (28.51- 36.01%). Rajapakse et al., (2010) revealed only 5% immature nut fall due to mealy bugs (*Dysmicocus* spp.), scale insects (*Aspidiotus destructor*), mites (*Dolichotetranychus* spp.), *Cyclodes omma* larvae and weevil (*Meridolus* spp.). In the present study, nut fall ranging from 22.33 to 36.01% was observed with medium to high incidence of *A. rugioperculatus* at both the locations. This drop was observed in spite of adoption of recommended package of practices. The dropping of nuts might be attributed to nutrient (nitrogen), protein, sugar content and water loss due to the sucking nature of *A. rugioperculatus* coupled with secretion of honey dew that facilitates the growth of sooty mould fungus (*Leptoxyphium* spp.) which further disrupts the photosynthetic ability (Josephraj et al., 2017). The favourable weather factors viz., minimum and maximum temperature and relative humidity play a vital role in incidence (Josephraj et al., 2018; Elango and Nelson, 2020). As the season progressed from November to March, the climatic conditions favoured the population buildup and consequent sap sucking resulting in more nutrient loss and resultant nut fall.

A minimal nut fall was observed during April to October, when incidence was absent; with Dr YSR HU - Godavari Ganga hybrid (T x D) drop was more than that of ECT palms which might be due to inheritance of susceptible characters of the dwarf parent. The dwarf coconut palms are more prone to heavy incidence compared to tall palms (Sundaraj and Selvaraj, 2017; Chandrika et al., 2017; Fousiya et al., 2019). Srinivasan et al. (2016) observed that dwarf palms suffered severe attack as compared to that of West Coast Tall and Arasampatti Tall. Selvaraj et al., (2016) observed that invasive *A. rugioperculatus* prefers to colonize on hybrid and dwarf varieties. Rajesh et al. (2020) observed more populations in dwarf Gautami Ganga followed by hybrids in Chhattisgarh. In the present study too, high incidence was observed in the Dr YSR HU- Godavari Ganga hybrid as compared to East Coast Tall (ECT). Kalavalapalli plantations revealed more nut fall compared to that of Dr YSR HU- HRS, Ambajipeta as intensity at Kalavalapalli was high due to highly suitable multicropping system of coconut and oil palm plantation. Similar findings were also reported by Kalidas (2019). The present observations on the yield loss are in contrast to those of Chandrika et al. (2017) and Subramanian et al. (2018) who stated that high incidence may not result in any economic loss in coconut. Perera (1993) reported heavy occurrence of pests causing over 40% damage to the coconut leaves during an outbreak of *O. arenosella*. Chandrika et al. (2010) recorded yield loss of 45.4% due to *O. arenosella.* Rajan et al. (2011) reported that

Table 1. Coconut dropped nuts (%) vs. *A. rugioperculatus* incidence- Dr. YSR HU- HRS, Ambajipeta/ Kalavalapalli (Mean± S.E)

Scale of incidence	Annual leaf production	No. of bunches	No. of buttons	Harvested nuts	Dropped nuts for 1 year	% of dropped nuts for 1 year (November 2020 to October 2021)	RSW incidence (November 2020 to March 2021)		No RSW incidence (April to October 2021)	
							Dropped nuts	% of dropped nuts for 6 months	Dropped nuts	% of dropped nuts for 6 months
Dr YSRHU-Godavari Ganga hybrid										
Ambajipeta										
Low (< 10 spirals/ leaflet)	13.80± 0.20	12.40± 0.24	160.80± 1.01	153.40± 0.71	7.80± 0.73	4.84± 0.43	5.20± 0.37	3.23± 0.23	2.60± 0.51	1.61± 0.31
Medium (10- 20 spirals/ leaflet)	13.20± 0.37	11.80± 0.37	157.80± 0.86	114.40± 1.72	43.60± 2.38	27.48± 1.35	29.60± 1.03	18.74± 0.55	13.80± 1.56	8.73± 0.98
High (> 20 spirals/ leaflet)	12.80± 0.37	11.20± 0.20	157.60± 1.81	102.00± 3.16	55.60± 1.86	35.32± 1.39	40.00± 1.00	25.40± 0.78	15.60± 0.93	9.92± 0.65
Kalavalapalli										
Low (< 10 spirals/ leaflet)	13.40± 0.24	12.00± 0.32	159.80± 1.02	151.00± 0.71	8.80± 0.73	5.50± 0.43	5.60± 0.51	3.49± 0.30	3.20± 0.37	2.00± 0.23
Medium (10- 20 spirals/ leaflet)	13.20± 0.37	11.60± 0.51	153.60± 1.63	110.40± 1.08	43.20± 1.07	28.11± 0.51	26.80± 0.86	17.46± 0.61	16.40± 1.03	10.66± 0.58
High (> 20 spirals/ leaflet)	13.00± 0.32	11.20± 0.37	158.20± 2.92	101.20± 1.80	57.00± 1.73	36.01± 0.67	39.60± 1.54	25.05± 0.97	17.40± 1.12	10.97± 0.53
East Coast Tall (ECT)										
Ambjipetta										
Low (< 10 spirals/ leaflet)	13.00± 0.44	11.60± 0.51	119.20± 3.03	112.80± 2.78	4.80± 0.73	4.06± 0.57	3.40± 0.51	2.88± 0.39	1.40± 0.24	1.18± 0.19
Medium (10- 20 spirals/ leaflet)	12.60± 0.24	11.20± 0.37	126.40± 1.57	98.20± 1.96	28.20± 1.16	22.33± 0.96	19.00± 0.71	15.04± 0.55	9.20± 0.58	7.29± 0.49
High (> 20 spirals/ leaflet)	12.00± 0.32	10.80± 0.58	127.00± 0.71	90.80± 0.96	36.20± 1.36	28.51± 0.95	24.00± 1.58	18.88± 1.19	12.20± 0.86	9.60± 0.66
Kalavalapalli										
Low (< 10 spirals / leaflet)	13.40± 0.40	11.80± 0.37	128.00± 3.71	122.00± 3.51	6.00± 0.45	4.68± 0.31	4.40± 0.51	3.44± 0.41	1.60± 0.24	1.24± 0.17
Medium (10- 20 spirals /leaflet)	12.60± 0.51	10.80± 0.37	116.80± 1.53	89.40± 2.09	27.40± 1.08	23.49± 1.06	19.80± 1.69	16.98± 1.52	7.60± 0.68	6.50± 0.56
High (> 20 spirals /leaflet)	12.00± 0.32	10.40± 0.51	119.20± 2.31	82.80± 2.44	36.40± 0.93	30.58± 0.95	26.40± 0.93	22.14± 0.64	10.00± 1.09	8.43± 1.01

slug caterpillar (*Macroplecta nararia* Moore) inflicts higher yield losses. Subramanian et al. (2018) reported that red palm weevil multiplies abundantly in young coconut plantations causing loss to an extent of 1-3%.

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AUTHOR CONTRIBUTION STATEMENT

All the authors have made a substantial contribution to the concept, design of the experiment and interpretation of data for the article. All the authors have read manuscript critically, involved in refining the article.

CONFLICT OF INTEREST

No conflict of interest.

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HABITAT ECOLOGY OF ASIAN KOEL *EUDYNAMYS SCOLOPACEUS* IN AGROECOSYSTEM

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ABSTRACT

The important role of birds in agriculture has long been recognized. The present study on the habitat, feeding and roosting behavior of Asian Koel (*Eudynamys scolopaceus*) in agro ecosystem of Punjab was carried from August 2021 to July 2022 at the Punjab Agricultural University (PAU), Ludhiana. It was conducted in three study areas i.e. study area A (agricultural field area), B (tree plantation area) and C (residential/ roadside area). The annual relative abundance of House Crow (*Corvus splendens*) was highest everywhere whereas of Asian Koel was 1%, 2.04% and 2.66% at study area A, B and C respectively. The feeding behavior of Asian Koel revealed that fruits and berries were preferred. It was found roosting mainly on Dhek, Neem, Peepal, Purple Bauhinia and Safeda trees. Asian Koel was most active during summer months and during the winters, it is not observed which might be because of its southward local migration.

Key words: Feeding, roosting, safeda, abundance, House Crow (*Corvus splendens*), line transect method, cuculiformes, omnivorous, fruits and berries, residential area, Common Myna (*Acridotheres tristis*)

Asian koel i.e., *Eudynamys scolopaceus*, of order cuculiformes measures about 38-46 cm and weighs 180-240 g (Choudhary, 2020). Asian koel shows sexual dimorphism with its conspecific. The behavioral practices show that it is generally omnivorous (Limparungpatthanakij, 2020). It is mainly known for its loud noise during its breeding season (Khan and Qureshi, 2020). Therefore, the present study was done because Asian koel is major concern due to its brood parasitic behavior with its different host species like house crow, common myna, black drongo (*Dicrurus macrocercus*) etc. as said by Nahid et al. (2016). The aim of the study was to explore the annual relative abundance of Asian koel with different bird species in three study areas of Ludhiana region on Punjab. Along with this, the feeding and roosting behavior of asian koel was also documented.

MATERIALS AND METHODS

The observations on Asian koel were carried for one year i.e. from August 2021 to July 2022 in Punjab Agricultural University (PAU), Ludhiana. The line transect and point count method (Kler and Kumar, 2015) was used for the study and the observations were taken twice a week, both during morning (6:30 to 8:30 am) and evening (4:30 to 7:30 pm). Identification of birds was done as per the keys given by Ali (2002). Standardized common and scientific names of the birds were described as per description given by Jayadevan et al. (2016). The observations on the annual relative abundance, feeding and roosting of Asian koel were made in three different study areas of PAU, Ludhiana viz. study area A (agricultural field area), study area B (tree plantation area) and study area C (residential/ roadside area). Abundant tree species were observed at study areas B and C as compared to study area A. The Mann-Whitney U test with software version SPSS16.0 was applied on comparative relative abundance of bird species of all the study areas. The study also categorizes bird species on the basis of their feeding habits as insectivorous (I), granivorous (G), fruits/ berries (F), plants/ aquatic vegetation/ nectar (P), small invertebrates (SI), small vertebrates/ fishes/ mice/ rat/ small birds/ eggs/ reptiles (SV) and omnivorous (O) as described by Ali (2002), on the basis of IUCN (2001) status for the categorization of birds as least concern (LC) and on the basis of preferred habitat as agricultural field area (A), tree plantation area (B) and residential/ roadside area (C).

RESULTS AND DISCUSSION

A total of 45 bird species were recorded out of which 40 bird species were found at study area A and B and 34 bird species were recorded at study area C.

Study area A and B supported maximum bird species which might be due to the presence of greater tree diversity. It had widespread agricultural lands and tree plantation/ forest/ orchard conditions. Mariappan et al. (2013) stated that majority of the birds depend upon their food from the agricultural and tree plantation areas. Easy food availability might be the reason of less interspecies competition observed at study area A and B. On the other side, study area C was recorded with least species diversity which might be due to area more inhabited by man.

The Asian koel showed maximum abundance at study area C (2.66%) followed by B (2.04%) and A (1.00%) which might be due to the presence of its host house crow which was maximum at study area C (25.12%) followed by A (25.05%) and B (20.08%). This can be reasoned due to the presence of greater opportunities of food due to human habitation. Therefore, difference in abundance from C to A of Asian koel was recorded as 1.66% (Table 1). It was also due to the fact that the variety in food items was greater at residential/ roadside area and tree plantation area for Asian koel as compared to agricultural field area. Also different types of vegetation of fruits and berries were present at B and C as compared to study area A. Researchers like Cushing et al. (2018) also stated that the factors like distribution, abundance and composition of bird species are often associated with gradients like vegetation cover and habitat availability. It was also found that despite of lowest species richness at C, the species like common moorhen, little brown dove, little egret and white wagtail were only known to be found at C and not at A and B. On contrary, species like Asian pied starling, black-winged stilt, barn swallow, house sparrow, Indian roller, paddy field pipit, pied bushchat, spotted munia, white-breasted kingfisher and white-breasted waterhen were known to be found at A and B and were absent at C. Not only this, bank myna was the only species that was found only at study area A.

The maximum annual relative abundance was that of house crow at all the study areas while the minimum annual relative abundance was of pied bushchat at A (0.09%) and B (0.14%) and of common moorhen at C (0.04%). The Mann-Whitney U test carried out to compare the relative abundance of bird species observed at all the locations gave Non-significant difference between relative abundance between study areas A and B, B and C and A and C at 5% level of significance. The foraging and feeding activities of Asian koel showed that it forages for the whole day irrespective of the daytime temperature. Adult koel was mainly observed to feed on trees with fruits. It was noted that it hopped from one branch to another, plucked the fruit and ate it. Sometimes two Asian koels were also observed to forage on the same tree. The observations on percentage of feeding items preferred by Asian koel were recorded and found that a total of five feeding items viz. nectar, seeds, fruits and berries, insects and dead animals were consumed by Asian koel. The data depicted that all types of food items were consumed by Asian koel in study area B and C. The percentage of fruits and berries consumed was highest as compared to the other food items at all the study areas and this might be due to the presence of tree diversity whereas it was less seen to feed upon dead animals which might be due to the reason that adult Asian koel feed mainly on fruits and only nestlings take protein rich dead arthropods as said by Corlett and Ping (1995). In addition to this, workers like Payne (2005) also recorded that Asian koel feeding habits consisted mostly of fruits especially nutmegs and banyan figs, insects, snail, flower and nectar. Not only this, he also observed Asian koel to feed upon eggs of other birds like bulbul and took small birds. Therefore, the present study and study by Payne in 2005 revealed almost same feeding habit of Asian koel.

The study on roosting behavior of Asian koel at different tree species was also done. The communal roosting in Asian koel was recorded on different tree species in different study areas. The roosting sites observed were directly proportional to the tree diversity present in that area. The roosting behavior of Asian koel was recorded high at some places whereas it was seen very low at some other places. It was also observed that they change their roosting site from one place to another within the same area. The roosting behaviour in study areas and on trees are given in Table 2. The maximum number of roosting sites i.e. 20 were observed at study area C followed by study area B and A with 15 and 6 roosting sites respectively. Tree species like dhek, neem, peepal, purple bauhinia and safeda were mainly utilized as roosting sites at all the study areas as compared to golden rain tree, jacaranda, kanak champa, kassod, mango, plum, satpatia, silver oak, tamarind and white mulberry tree species that were least utilized. The present study revealed that the more number of roosting sites might be due to presence of human habitation and abundance in tree species as compared to other study areas. Whereas Begum et al. (2011) in their work showed that Asian koel roosts mainly upon fruit trees and recorded trees like *Ficus bengalensis*, *Psidium guajava*, *Ficus racemosa*, *Syzygium jambos*,

Table 1. Annual abundance and other details of Asian koel

S. No.	Bird species (Common name)	Bird species (Scientific name)	Annual abundance			Food	IUCN status	Habitat type
			A	B	C			
1	Asian koel	*Eudynamys scolopaceus*	1	2.04	2.66	I, F	LC	ABC
2	Asian pied starling	*Sturnus contra*	2.56	2.91	-	I, F	LC	AB
3	Bank myna	*Acridotheres ginginianus*	5.35	-	-	I, F	LC	A
4	Barn swallow	*Hirundo rustica*	2.2	2.99	-	I	LC	AB
5	Black drongo	*Dicrurus macrocercus*	1.93	1.31	1.11	I	LC	ABC
6	Black kite	*Milvus migrans*	5.1	3.21	5.42	I, R	LC	ABC
7	Black-winged stilt	*Himantopus himantopus*	0.63	0.7	-	I	LC	AB
8	Brown rock chat	*Cercomela fusca*	-	0.25	0.18	I	LC	BC
9	Cattle egret	*Bubulcus ibis*	2.91	2.59	0.7	I, SI	LC	ABC
10	Common hoopoe	*Upupa epops*	0.27	0.35	0.45	I	LC	ABC
11	Common moorhen	*Gallinula chloropus*	-	-	0.04	I, SI, G, P	LC	C
12	Common myna	*Acridotheres tristis*	4.61	9.87	12.53	I, F	LC	ABC
13	Coppersmith barbet	*Megalaima haemacephala*	0.15	0.27	0.09	I, F	LC	ABC
14	Eurasian collared dove	*Streptopelia decaocto*	2.98	3.72	4.08	G	LC	ABC
15	Greater coucal	*Centropus sinensis*	1.5	1.43	1.56	I, SI, SV	LC	ABC
16	Green bee-eater	*Merops orientalis*	2.69	4.42	0.72	I	LC	ABC
17	Grey francolin	*Francolinus pondicerianus*	1.12	1.56	0.11	G, I	LC	ABC
18	House crow	*Corvus splendens*	25.05	20.08	25.12	O	LC	ABC
19	House sparrow	*Passer domesticus*	0.5	0.57	-	G, I	LC	AB
20	Indian black ibis	*Pseudibis papillosa*	0.97	0.69	0.94	G, I	LC	ABC
21	Indian grey hornbill	*Ocyceros birostris*	0.67	0.81	1.14	F, I	LC	ABC
22	Indian peafowl	*Pavo cristatus*	0.72	1.93	0.72	G, P, I, SV	LC	ABC
23	Indian roller	*Coracias benghalensis*	0.19	0.2	-	I	LC	AB
24	Jungle babbler	*Turdoides striatus*	5.74	6.24	9.78	I,F	LC	ABC
25	Lesser golden-backed woodpecker	*Dinopium bengelensis*	0.21	0.34	0.35	I	LC	ABC
26	Little brown dove	*Streptopelia senegalensis*	-	-	1.99	G	LC	C
27	Little egret	*Egretta garzetta*	-	-	0.07	I, SI	LC	C
28	Oriental magpie-robin	*Copsychus saularis*	0.49	0.56	0.79	I	LC	ABC
29	Paddy field pipit	*Anthus rufulus*	2.12	0.82	-	I, P	LC	AB
30	Pied bushchat	*Saxicola caprata*	0.09	0.14	-	I	LC	AB
31	Purple sunbird	*Nectarinia asiatica*	0.49	0.87	1.1	P	LC	ABC
32	Red-vented bulbul	*Pycnonotus cafer*	2.43	1.97	2.55	I, P, F	LC	ABC
33	Red-wattled lapwing	*Vanellus indicus*	3.25	2.9	4.4	I, SI	LC	ABC
34	Rock pigeon	*Columba livia*	5.04	8.6	7.46	G	LC	ABC
35	Rose-ringed parakeet	*Psittacula krameri*	5.46	5.52	8.77	F, P, G	LC	ABC
36	Rufous treepie	*Dendrocitta vagabunda*	0.74	0.68	0.65	I, SV	LC	ABC
37	Shikra	*Accipiter badius*	1.06	1.61	0.99	I, SV	LC	ABC
38	Spotted munia	*Lonchura punctulata*	4.26	0.3	-	I, G	LC	AB
39	Spotted owlet	*Athene brama*	0.3	0.39	0.22	I, SV	LC	ABC
40	White wagtail	*Motacilla alba*	-	-	0.1	I, SI	LC	C
41	White-breasted kingfisher	*Halcyon smyrnensis*	0.51	0.46	-	I, SV	LC	AB
42	White-breasted waterhen	*Amaurornis phoenicurus*	0.35	0.8	-	I, SI, G, P	LC	AB
43	White-throated munia	*Euodice malabarica*	0.58	0.28	0.58	G, P	LC	ABC
44	Wire-tailed swallow	*Hirundo smithii*	3.12	4.39	0.11	I	LC	ABC
45	Yellow-legged green pigeon	*Treron phoenicoptera*	0.68	1.24	2.53	F	LC	ABC

Table 2. Roosting sites of Asian koel

Tree species \ Study area	A	B	C
Amaltas	x	√	√
Ashoka	x	√	√
Banyan	x	√	√
Dhek	√	√	√
Golden rain tree	x	x	√
Gulmohar	x	√	√
Indian gooseberry	x	√	√
Ipil-Ipil	√	√	x
Jacaranda	x	x	√
Kanak Champa	x	x	√
Kassod	x	x	√
Mango	x	√	x
Neem	√	√	√
Peepal	√	√	√
Plum	x	x	√
Purple Bauhinia	√	√	√
Safeda	√	√	√
Satpatia	x	x	√
Silver Oak	x	x	√
Spotted Fig	x	√	√
Tahli	x	√	√
Tamarind	x	√	x
White mulberry	x	x	√
Total	6	15	20

√: utilized as roosting site by Asian koel; x: not utilized as roosting site by Asian koel

Eugenia javanica, *Zizyphus mauritiana*, *Carica papaya* and *Polyalthia longifolia*. Therefore both the studies showed some variations in roosting sites of Asian koel. Therefore, the results regarding abundance, feeding and roosting sites of Asian koel revealed that it preferred areas mainly with good tree diversity. The presence of human habitation and abundance of house crow which act as a host for Asian koel, was some key priority areas for the Asian koel. The coevolution of host parasite relationship turn this study towards its future perspectives that can mention the evolutionarily interspecific dependence of Asian koel with house crow and other host species for its breeding (Jayadevan and Lowther, 2020).

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AUTHOR CONTRBUTION STATEMENT

The designing of the research problem was done by M K and the execution and observations were made by M J. Both the authors interpreted the data. M J wrote the manuscript and M K read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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SEASONAL INCIDENCE OF *SPODOPTERA LITURA* (F) ON SOYBEAN

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ABSTRACT

An investigation was carried out to "Study on seasonal incidence of *Spodoptera litura* (F.) on soybean" at Agriculture Research Station (ARS), Adilabad, Professor Jayashankar Telangana State Agricultural University during kharif, 2017 and 2018. The pooled results indicated that among different sowing dates viz., 15th-18th of June (sowing I), 25th-28th of June (sowing II), 5th-7th of July (sowing III), 15th -17th of July (sowing IV) and 25th-27th of July (sowing V) sowing I recorded minimum incidence (2.81 larvae/ meter row length) of *S. litura* population and maximum incidence in sowing II (4.34 larvae/ meter row length), similarly sowing I recorded minimum incidence (2.97 larvae/ meter row length) and maximum incidence in sowing II (4.34 larvae/ meter row length) during kharif, 2017 and 2018, respectively.

Key words: Seasonal incidence, *Spodoptera litura*, soybean, Kharif, sowing dates, minimum incidence, population.

In India, soybean is cultivated in an area of 113.98 lakh ha. during 2019-20 (SOPA., 2020). Nearly 48% of soybean cultivated area is contributed by Madhya Pradesh and is called as 'Soya-state". Previous researchers' findings concluded that seed yield and seed quality are being adversely affected by major insect pests viz., girdle beetle, tobacco caterpillar, green semi looper, jassids and whitefly (Ahirwal et al., 2015). The infestation varied with date of sowing (Kalyan and Ameta, 2017). Understanding the fluctuations in weather parameters over a period of time and adjusting the management practices towards achieving better yields for improvement of social economic status. Hence the study was carried out to know the damage of various insect pests of soybean in relation to weather parameters.

MATERIALS AND METHODS

The present experiment was carried out at Agriculture research station, Adilabad, during kharif, 2017 and 2018. Adilabad is situated in Northern zone of Telangana state (19º 40 12.00" N, 78º 31 48.00" E with an altitude of 264 m from Mean Sea Level (MSL). The experimental field represented medium black soil with good drainage and possessed low to medium organic matter as well as potash contents. The pH and soluble salts were normal. Fertilizers were applied at the time of sowing as basal dose of 30 kg N, 60 kg P_2O_5 and 40 kg K_2O/ ha in the form of Urea, Diammonium phosphate and Murate of potash, respectively.

Soybean, variety JS 335 was sown in an area of 1250 m^2 with a plot size of 50 x 5 m. Five such sowings were taken up on different dates during kharif, 2017 viz., on 18.06.17, 28.06.17, 07.07.17, 17.07.17 and 27.07.17 and similar sowing were taken up during kharif, 2018 on 15.06.18, 25.06.18, 05.07.18, 15.07.18 and 25.07.18. Spacing of 45 x 5 cm was followed between rows and plants of soybean. The experiment was conducted under unprotected conditions. The data obtained were analysed and transformed to corresponding angular transformed values with one factor analysis with OPSTAT Statistical Software Package of Chaudhary Charan Singh Haryana Agricultural University, Hisar.

The observation on tobacco caterpillar (No. of larvae/ meter row length) was recorded once in a standard week at three locations of one meter row length (meter row length) from vegetative stage of the crop till harvesting stage of the crop.

RESULTS AND DISCUSSION

The incidence of *S. litura* in soybean crop was observed in all the dates of sowing during kharif, 2017 where it started during 28th Standard Meteorological Week (1.10 larvae/ meter row length). The population increased gradually and reached peak during 36th SMW (4.30 larvae/ meter row length). Similarly, during sowing II, the incidence started from 29th SMW (0.40 larvae/ meter row length) with an overall larval population ranging from 0.40 to 7.80 larvae/ meter row length. Likewise in the sowings III, IV and V the larval population ranged between 1.82 to 6.24, 1.00 to

Table 1. Seasonal incidence of *S. litura* (F.) on soybean

Sowing I		Sowing II		Sowing III		Sowing IV		Sowing V	
SMW	Larva (No./ meter row length)	SMW	Larva (No./ meter row length)	SMW	Larva (No./ meter row length)	SMW	Larva (No./ meter row length)	SMW	Larva (No./ meter row length)
Kharif, 2017									
28	1.10	29	0.40	30	2.86	31	1.10	32	1.90
29	1.52	30	1.60	31	6.14	32	1.52	33	2.20
30	1.90	31	1.60	32	6.24	33	1.90	34	2.50
31	2.85	32	2.20	33	5.32	34	2.85	35	2.65
32	2.92	33	4.80	34	5.21	35	2.92	36	3.20
33	3.20	34	5.40	35	4.20	36	3.20	37	3.54
34	3.24	35	4.20	36	3.80	37	3.24	38	2.52
35	4.21	36	6.60	37	3.40	38	4.21	39	2.20
36	4.30	37	6.60	38	2.80	39	4.30	40	1.90
37	2.90	38	7.80	39	2.44	40	2.90	41	1.70
38	2.80	39	6.54	40	1.82	41	1.00	42	1.04
Mean	2.81	Mean	4.34	Mean	4.02	Mean	2.65	Mean	2.30
Kharif, 2018									
28	0.90	29	0.50	30	0.84	31	0.95	32	0.52
29	1.25	30	1.56	31	1.24	32	1.00	33	1.25
30	1.40	31	1.78	32	1.36	33	1.20	34	1.80
31	2.00	32	2.00	33	1.58	34	1.40	35	2.12
32	2.56	33	2.45	34	1.94	35	2.00	36	2.40
33	2.58	34	3.20	35	2.16	36	2.15	37	2.63
34	3.00	35	3.95	36	3.54	37	3.00	38	3.50
35	3.90	36	4.50	37	4.54	38	3.20	39	3.90
36	4.20	37	5.20	38	4.62	39	4.00	40	2.68
37	5.00	38	5.00	39	5.42	40	4.20	41	4.20
38	5.90	39	5.60	40	5.90	41	5.00	42	4.25
Mean	2.97	Mean	3.25	Mean	3.01	Mean	2.55	Mean	2.68

(Kharif 2017, 2018)

Treatments	Larva (No./mrl)	Treatments	Larva (No./mrl)
(Sowing I) 18-06-17	2.81 (1.97)	(Sowing I) 15-06-18	2.97 (1.99)
(Sowing II) 28-06-17	4.34 (2.33)	(Sowing II) 25-06-18	3.25 (2.07)
(Sowing III) 07-07-17	4.02 (2.25)	(Sowing III) 05-07-18	3.01 (2.01)
(Sowing IV) 17-07-17	2.65 (1.91)	(Sowing IV) 15-07-18	2.55 (1.89)
(Sowing V) 27-07-17	2.30 (1.83)	(Sowing V) 25-07-18	2.68 (1.92)
SE m±	0.007	SE m±	0.003
CD at 5%**	0.02	CD at 5%**	0.008
CV %	0.718	CV %	0.292

mrl = meter row length; Figures in parenthesis angular transformed values

4.30 and 1.04 to 3.54 larvae/ meter row length with the highest larval population during 32nd SMW (6.24 larvae/ meter row length), 39th SMW (4.30 larvae/ meter row length) and 37th SMW (3.54 larvae/ meter row length), respectively (Table 1).

During kharif, 2018, the incidence of *S. litura* ranged between 0.90 to 5.90, 0.50 to 5.60, 0.84 to 5.90, 0.95 to 5.00 and 0.52 to 4.25 larvae/ meter row length with the highest population during 38th SMW (5.90 larvae/ meter row length), 39th SMW (5.60 larvae/ meter row length), 40th SMW (5.90 larvae/ meter row length), 41st SMW (5.00 larvae/ meter row length) and 42nd SMW (4.25 larvae/ meter row length) during sowings I, II, III, IV and V, respectively.

Among different dates of sowings, the incidence of *S. litura* population was low in sowing I (2.81 larvae/ meter row length) followed by gradual increase in II (4.34 larvae/ meter row length) and III (4.02 larvae/ meter row length) sowings which showed decrease in IV (2.65 larvae/ meter row length) and V (2.30 larvae/ meter row length) sowings. However, during kharif, 2018 the lowest incidence of *S. litura* population was recorded in sowing IV (2.55 larvae/ meter row length) followed by sowing V (2.68 larvae/ meter row length), sowing III (3.01 larvae/ meter row length) and sowing I (2.97 larvae/ meter row length), respectively, though maximum mean incidence of 3.25 larvae/meter row length was recorded in sowing II. From the above results it is concluded that minimum incidence of pest population was observed in sowing I.

The present findings are in partial agreement with the findings of Harish et al. (2008) who reported that maximum larval population of *S. litura* (7.80, 6.50, and 8.60 larvae/ meter row length, respectively) was noticed on the crop sown on 08-06-06, 27-06-06 and 08-07-06 dates, respectively revealing early sown crop recording lower incidence of *S. litura*. Basic information on the seasonal incidence of leaf eating caterpillars on soybean is considered as most essential to manage the pest in soybean. Mandal et al., (1998) observed *S. litura* being low in early sown (22nd June and 2nd July) soybean. Incidence of this pest was high in the crop when sown between 12th July and 1st August. Yeotikar et al., (2015) reported that the incidence of *S. litura* was observed during 32nd to 35th SMW. Babu et al., (2015) recorded the incidence from 30th SMW to 38th SMW, whereas, Matti and Deotale et al. (2017) reported incidence of *S. litura* from 35th to 46th SMW. Babu et al., (2015) also found that, adult population of *S. litura* was active from August to mid-October which later decreased sharply in late October. The peak appearance was observed during September-October months and corresponded with peak activity of egg masses and larval population in soybean contributed to the outbreak of the pest during reproductive stage of the crop.

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AUTHOR CONTRIBUTION STATEMENT

K R S and S C framed idea and designed the study. S K collected the samples. K R S and S C wrote and edited the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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MOLECULAR TOXONOMY OF FORENSICALLY IMPORTANT CARRION FLIES USING mt (DNA COI)

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ABSTRACT

Identification of forensically important carrion flies is made challenging by the lack of taxonomists and morphological identification keys. The eggs or larvae are difficult to identify morphologically, and in such cases, molecular technique, DNA Barcoding specific to mt (DNA COI) provides a genuine substitute. The mtCOI of two Calliphoridae species, *Chrysomya megacephala, Chrysomya rufifacies* and one Sarcophagidae species, *Sarcophaga dux* are examined in the present study for molecular identification.

Key words: Molecular identification, *Chrysomya megacephala*, *Chrysomya. rufifacies*, *Sarcophaga dux*, forensic, maggots, carrion flies, mtDNA.

Forensic entomologists use arthropods to determine the postmortem interval (PMI) and the timing of the earliest insect colonisation of humans or other animals. The assessment of PMI can be aided by insect evidence found at crime scenes (Byrd and Tomberlin, 2019). The perinatal stages of insects are often related to species identification, which can be challenging because larval features alone do not provide precise and sufficient identification keys. According to Byrd and Castner (2010), many of these prenatal stages in various animals are yet unknown. Only a few species may be identified using morphological identification keys (Velasquez et al., 2010). As an alternative to morphological identification, mtDNA sequence data can be used to identify immature forms of the species (Hebert et al., 2003). DNA barcoding was suggested by Kress et al. (2005) for accurate identifications of species. One of the first taxonomists to make considerable use of DNA sequences for identifying and defining species was the Dipteran taxonomist. For Dipterans, a variety of molecular markers are used (Wells and Stevens, 2008). A forensic entomologist can benefit from molecular analysis to correctly identify entomological evidence (Bharati and Singh, 2017). The present study focused on the few Calliphoridae and Sarcophagidae flies of Osmanabad, Maharashtra, India.

MATERIALS AND METHODS

A fresh liver sample was purchased from a local slaughterhouse, and when putrefied, it was exposed to air to attract flies. Various animal remains were also used to collect the larvae and adult flies. An insect-catching net was used to catch flies. Flies were collected, brought to the laboratory, raised, and fed diluted honey and raw liver. Fresh liver was used as an oviposition site for females. Until the post-feeding phase, the maggot cultures were fed raw liver. The pupae were placed in 500 ml beakers with 2 cm of dry soil. The adult flies were raised in a rearing box (25x 25x 45 cm) and pure culture was established by separating the eggs or larvae of one female for identification and other experiments. The Magnus MS-224 stereoscopic microscope was used to morphologically analyze these adults and maggots. Several stages of Calliphoridae and Sarcophagidae species were dissected and identified using morphological traits and previously published identification keys. For molecular analyses the samples 1 = *Sarcophaga dux*, 4 = *Chrysomya megacephala*, and 14 = *Chrysomya rufifacies* each were used providing a unique sample ID. DNA barcoding was performed on the thorax of an adult flies. The ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with (FS enzyme) AmpliTaq®DNA polymerase, Montage PCR cleanup kit, QIAamp® DNA small genomic isolation kit, and ABI 3730xl sequencer were used (Applied Bio-Systems). DNA sequencing programs for blasting and alignment (Muscle 3.7, Mega Blast software, Gblocks 0.9b, PhyML 3.0 aLRT, ClustalX v2.012, and Bio Edit Sequence Alignment Editor V7.0.5.3) were used. LCO-1490 GGTCAACAAATCATAAAGATATTGG. 25 base pairs and HCO-2198 T 26 base pairs primers were used. The NCBI blast similarity search tool was used to look up the

18S RNA sequence, and following phylogenetic analysis, closely related sequences, and multiple sequences were performed. The Muscle 3.7 programs were used to align many sequences (Edgar, 2004). Gblocks 0.91b was used to process the aligned sequences that were generated. This G block reduces alignment noise by removing diverging and uneven alignments (Talavera and Castresana, 2007). Finally, PhyML 3.0 aLRT and the HKY85 substitution models were used to conduct phylogenetic analysis and TreeDyn 198.3 was employed to render the trees (Dereeper et al., 2008).

RESULTS AND DISCUSSION

GenBank accession numbers were obtained for each sample of *C. megacephala* (MG816777), *C. rufifacies* (MG816778), and *Sarcophaga dux* (MG816779). The mean and maximum intraspecific values for each species are compared to their closest relatives- *C. megacephala* is closest to *S. dux, C. rufifacies* is closest to *C. megacephala,* and *C. rufifacies* to *C. megacephala.* The closest species distance given by *C. megacephala* and *C. rufifacies* is 7.13, whereas *S. dux* indicates a distance of 11.49. While the maximum intraspecific distance was zero, the mean intraspecific distance did not apply to any of the three forensic flies. Sequence analysis using a species database showed a 100% match to the *C. megacephala* species, which was identified visually as sample ID 4. A=30.4%, T=37.4%, G=16.0%, and C=16.2% make up the nucleotide frequency distribution of *C. megacephala.* Sequence analysis using a species database showed a 100% match to the *C. rufifacies* species, which was identified visually as sample ID 14. The mean nucleotide composition frequency of *C. rufifacies* was A = 30.4%, T = 38.1%, G =15.8%, and C =15.7%. This work led to the discovery of the genus *Sarcophaga*, which is where the recovered sarcophagid sample from a species identified morphologically as *S. dux* and given sample ID number 1 comes from. The top 99 species in a species database used to identify sequences showed a 100% match to *S. dux*. Within the *S. dux* genus, the mean nucleotide frequency distribution is A = 30.8%, T = 37.4%, G = 16.1%, and C = 15.6%.

Phylogenetic analysis of *C. megacephala*, *C. rufifacies,* and *S. dux* shows 100% matches (Figs. 1-3). Sukontason et al. (2003) concluded that *C. rufifacies* and *C. megacephala* have strong similarities despite using scanning electron microscopy, and DNA barcoding is a valuable tool. Many forensically significant dipteran species' eggs or larvae are extremely difficult to differentiate morphologically (Benecke and Seifert, 1999, Liu and Greenberg, 1989). This is owing to the fact that adult arrival timings, egg length, and larval growth rates vary greatly between species. Also, keys are only relevant to a small number of species, the publication of identification keys is based on larval morphology. Sperling et al. (1994) were the first to demonstrate how mtDNA sequencing data from adult specimens of forensically significant flies may be used to distinguish between juvenile members of the same species. Wells and Sperling (1999) discovered using the same methods

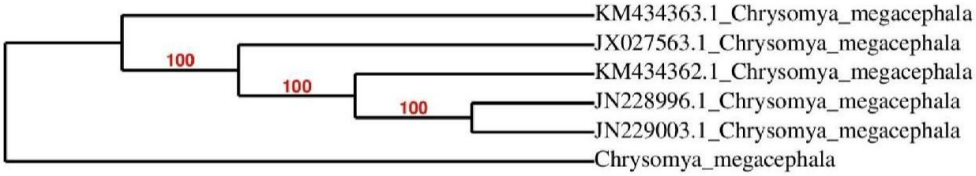


Fig. 1. Phylogenetic tree of *C. megacephala*

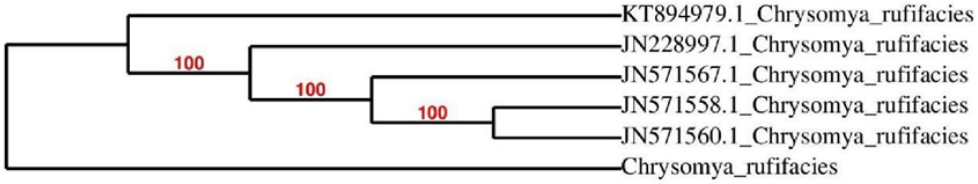


Fig. 2. Phylogenetic tree of *C. rufifacies*

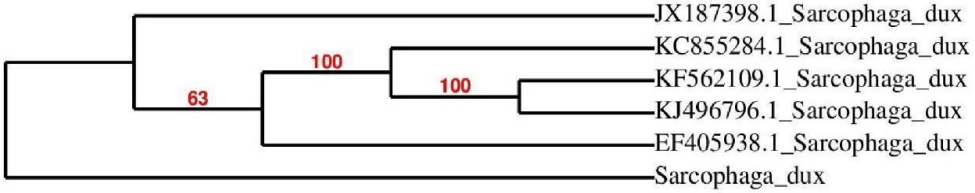


Fig. 3. Phylogenetic tree of *S. dux*

that Calliphoridae species that can be challenging to distinguish taxonomically even in the adult stage have distinctly different mtDNA. Hebert et al. (2003) proposed DNA barcoding, which uses mtDNA to identify unknown using a reference library of categorized specimens (Kress et al., 2005). The mitochondrial COI gene, which is the standard region for DNA barcoding, has the best taxon coverage of all the molecular markers that have been utilized in Diptera (Wells and Stevens, 2008). According to Dawnay et al. (2007), an efficient method for identifying species within the Calliphoridae subfamilies appears to be the examination of the mtDNA. The advantages of mtDNA over nuclear DNA extend beyond this. mtDNA is crucial for molecular identification because it can reveal variations in sequences between closely related species (Waugh, 2007).

This study presents the genetic identification of two Calliphorid species using DNA barcoding. The cytochrome C oxidase subunit I (COI) gene was used to establish the physical differences between two calliphorid species. *Chrysomya's* mean nucleotide frequency distribution was A = 30.4%, T = 38.1%, C = 15.8%, and G = 15.6%, according to Nelson et al. (2007). According to Bajpai et al. (2013), the nucleotide composition was T = 39.6%. A = 31.4%, C = 15.4%, G = 14%. Regional dispersal causes a small but not very noticeable variation in the nucleotide composition. Ullerich and Schöttke (2006) assert that *C. megacephala* and *C. saffranea* share physical and genetic characteristics, but differ ecologically. The physical differences between *C. saffranea* and *C. megacephala* are minimal (Spradbery, 2002), but when species were identified using the COI gene, no interspecific sequences were found, indicating that *C. saffranea* and *C. megacephala* are monophyletic. The highest interspecific sequence variation between *C. saffranea* and *C. megacephala,* according to Harvey et al. (2008) utilizing NO3RD analysis, was only 0.33%, indicating *C. megacephala* and *C. saffranea* species are related. Once more, Harvey et al. (2008) demonstrated that the highest intraspecific variation of *C. saffranea* and *C. megacephala* was 0.18% and 0.34%, respectively, and that the interspecific variance between *C. megacephala* and *C. saffranea* only varied by 0.23%. (Proportion of the 1167 base pairs total for COI) (Abd Al Galil, 2015). His opinion of COI barcoding of *C. megacephala* showed that the nucleotide composition of the genus *Chrysomya* was G = 15%, C = 15.5%, A = 30.85%, and T = 38.3% on average.

The genus Sarcophaga was represented by one member of the Sarcophagidae family, *S.dux*. According to Bajpai and Tewari (2010), the nucleotide composition of all collected Sarcophagidae species was T = 40%, A = 31%, C = 15%, and G = 14%. Meiklejohn et al. (2011) showed that the Australian Sarcophagidae have an A-T bias in their nucleotide composition (mean A = 29.66%, T = 37.02%, C = 17. 43, G = 15.89%). According to Kimura's 2-parameter model, the sequence divergence in the COI barcode region among these sarcophagidae at the species and family levels was 0.0% (K2P). The ranges of sequence divergence at the level of the genus, however, were as follows: Minimum distance = 7.87%, maximum distance = 8.58%, and mean distance. = 8.22%, and standard error = 0.1%. The distribution of sequence divergence, however, was minimum distance = 7.45%, maximum distance = 8.05%, mean distance = 7.75%, and standard error = 0.08%, according to the pairwise distance the distribution of sequence divergence was minimum distance = 7.45%, maximum distance = 8.05 %, mean distance = 7.75% and, the standard error = 0.08%.

The minimum, mean, and maximum distances to the nearest neighbor in the Barcode Gap study of the distribution of distances within each species and the distance to each species' nearest neighbor were 7.45%, 7.55%, and 7.75%, respectively, and the SE was 0.05. According to Bajpai and Tewari (2010), the distance between the two species was 9.4%, but the pairwise difference parameter revealed a distance of 7.45% between the barcode species *S. dux* and its nearest neighbor species *S. ruficornis*. The size difference between *S. peregrine* and *S. ruficornis*, the most closely related species, was 7.75%. The phylogenetic tree revealed that *S. ruficornis* and *S. dux* share the majority of common ancestor features. Out-groups of S. peregrine include *S. ruficornis* and *S. dux*. The Sarcophaga genus had a mean nucleotide composition frequency distribution of G = 15.85%, C = 16.31%, A = 29.99%, and T = 37.84%, according to % Abd Al Galil (2015). A value of 100% matches in this database's BOLD search suggests matches with a high level of confidence, whereas a value of fewer than 99% matches indicates a low level of matching.

The flies collected in this study form the basis for baseline data regarding the collection of local carrion flies and their use in forensic sciences. Molecular analyses suggest the use of DNA barcoding as a practical method for identifying fly species. However, the success of this method depends on the upkeep of a comprehensive and reliable genetic reference catalog in GenBank or other readily available data aggregation sources. Identification

of entomological species, a vital step in forensic investigations, can occasionally be difficult simply based on morphology. By using molecular techniques, species or genera that share a similar morphology can be quickly distinguished and are particularly useful since they can be used at any stage of life. The use of DNA-based sequence data can precisely identify an insect's species, overcoming the drawbacks of morphology-based identification.

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CONFLICT OF INTEREST

No conflict of interest.

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AUTHOR CONTRIBUTION STATEMENT

SB designed and carried out the entire study, and VM supervised it by providing timely guidance. SB analyzed data, and VM assisted in data compilation. SB wrote the manuscript, and VM and VR read, corrected, and approved it.

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BASELINE SUSCEPTIBILITY OF RED SPIDER MITE *TETRANYCHUS URTICAE* KOCH ON TOMATO TO SELECTED ACARICIDES

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ABSTRACT

This study was conducted to establish the baseline susceptibility data for *Tetranychus urticae* Koch, successfully maintained under laboratory conditions without exposure to acaricides. The leaf dip method of bioassay was used to establish the baseline susceptibility. Toxicity (LC50 value) varied across the major acaricides i.e., chlorfenapyr 10SC, propargite 57EC, fenazaquin 10EC, abamectin 3EC, spiromesifen 22.9SC and dicofol 18.5EC. Maximum toxicity was recorded for fenazaquin 10EC (0.08 ppm), followed by propargite 57EC, and the lowest was recorded with chlorfenapyr 10 SC (0.16 ppm). The LC_{50} values for all acaricides tested were lower than concentrations used as recommended field doses. This baseline data might be useful in monitoring and in the management of acaricide resistance in *T. urticae*.

Key words: Acaricides, bioassay, LC_{50}, baseline, susceptibility, *Tetranychus urticae*, mortality, fiducial limit, mulberry leaves, resistance, fenazaquin, propargite, chlorfenapyr

In recent years, mites have become serious pests and are gaining importance owing to their devastating nature and severe damage potential. Globally more than 1,200 species of spider mites have been identified (Bolland et al*.,* 1998; Migeon et al., 2010). Among them, the red spider mite, *T. urticae* Koch, is a predominant one and causes considerable damage to crops, particularly tomato, cowpea, french bean, okra, and brinjal (Singh and Raghuraman, 2011). These mites cause a significant yield loss of 26.94 to 64.93 per cent in solanaceous crops (Shukla et al., 2017). The control of red spider mites is usually accomplished by the application of acaricides (Roy, 2019). Although many non-chemical control tactics are available, farmers still rely on chemical pesticides to manage pests (Veres, 2020). The indiscriminate use of fertilizers, monocropping, and the application of synthetic pesticides have generated favourable conditions that allowed *T. urticae* populations to multiply and cause epidemics in agricultural systems (Ramasubramanian*,* 2005, Leeuwen et al*.,* 2010). Acaricide resistance in phytophagous mites is on the rise, particularly in *T. urticae*, which have a high potential for rapid resistance evolution (Badieinia, 2020). Furthermore, their high rate of multiplication, short life cycle, polyphagous nature, and high dispersal contribute to the quick resistance development (Kumar and Manmeet, 2018).

The widespread and indiscriminate use of acaricides to control *T. urticae* has resulted in the emergence of resistant populations in over 40 countries (Georghiou and Tejeda, 1991) and resistance to at least 95 different acaricides. More than 600 species of insects and mites have developed resistance to at least one class of insecticides /acaricides (Sparks et al., 2020). Chemical insecticides used to control red spider mites in modern agriculture are becoming ineffective and expensive due to the rapid development of resistance to most pesticides (Kumar, 2011). Moreover, baseline data on the toxicity of acaricides would greatly help in understanding the potentiality of the mite species to develop resistance to acaricides, which is the reference values for all comparisons and assessment of intensities of resistance (Sharma, 2018). Baseline data determined in the current study is the highest susceptibility level of *T. urticae* to a target acaricide when it is fully devoid selection pressure and is suggested to use it for determining the level or extent of resistance to an acaricide (Noor and Srinivasa, 2020). Because of this, the present investigation has been planned and designed

to determine the susceptibility of red spider mites to selected acaricides.

MATERIALS AND METHODS

The present investigations on the establishment of baseline susceptibility of red spider mite, *Tetranychus urticae* Koch, to selected acaricides were carried out in the Department of Entomology, College of Horticulture, University of Horticultural Sciences, GKVK campus, Bengaluru, Karnataka during 2020-21 (12°.58' N and 77°.38' E). The susceptible laboratory culture (171st generation) of two-spotted spider mite, *T. urticae* was collected from the All India Network Project (AINP) on Agriculture Acarology, Department of Entomology, UAS, GKVK, Bengaluru and maintained on mulberry leaves under laboratory condition at ambient room temperature 27± 2°C and 75± 5% relative humidity without exposure to any acaricides.The median lethal concentration (LC_{50}) values determined for the susceptible laboratory culture of *T. urticae* served as the baseline values. The leaf dip bioassay method was adapted to determine the baseline susceptible values as recommended by the Insecticide Resistance Action Committee (IRAC) (Roy et al., 2010). Before fixing the concentration for bioassay, preliminary bioassay (bracketing assay method) was performed to fix the concentrations of each acaricide, giving 10 to 90 per cent mite mortality.

The collected colonies were reared on detached fresh mulberry leaves, placed on wet polyethylene foam, and kept in a plastic tray in the laboratory to get a sufficient population (F1 or F2 generation) for bioassay. The leaf dip bioassay method was adapted to test the toxicity of six acaricides against red spider mites namely propargite, spiromesifen, fenazaquin, abamectin, chlorfenapyr, and dicofol. After conducting the preliminary bioassay resulting in 10 to 90% mortality, the actual dose of seven concentrations was fixed for further experimentation. For conducting the leaf dip bioassay, fresh mulberry (*Morus alba* L.) leaves of uniform size and age were cut into 4 cm diameter discs. Each leaf disc was dipped in appropriate acaricide concentration for 10-15 seconds and were placed ventral surface down, individually on a water-saturated cotton pad (9 x 9 cm) in the petri plates (10 cm diameter x 1.5 cm height). Thirty *T. urticae* adults were transferred to each treated leaf disc with a fine camel hair brush. Three replications were maintained and the leaf disc treated with water was used as control. Observations on mite mortality were recorded at 24 h, 48 h, and 72 h after treatment by using a stereo-binocular microscope and fine camel hair brush. The data recorded on mortality were subjected to probit analysis (Finney, 1971) using SPSS v.16 software after correcting the mortality in control by following the Abbott formula (Abbott, 1925). During the observation, mites that were alive but could not move when softly probed with a delicate hair brush were considered as moribund or dead.

RESULTS AND DISCUSSION

To generate baseline susceptibility data (LC50 values), six acaricides were tested with three replications against the susceptible laboratory population of red spider mites, *T. urticae*. Based on the LC50 values obtained by the leaf dip method against red spider mites, *T. urticae* proved to be highly susceptible to fenazaquin 10 EC with an LC50 value of 0.08 ppm. Fenazaquin 10 EC showed a non-significant difference with overlapping fiducial limits (0.070-0.180 ppm). Noor and Srinivasa (2019), found an LC50 value of 0.23 ppm for Fenazaquin 10 EC during the 60th generation and the LC_{50} of 0.22 ppm against susceptible laboratory population at 91st generation on tomato crop (Noor and Srinivasa, 2020; Mohin et al., 2021). The susceptibility of red spider mite population to propargite 57 EC, (0.094 ppm) was next to Fenazaquin 10 EC. There was no significant difference between six acaricides (0.080-0.180 ppm) with overlapping fiducial limits. Mohin et al. (2021) reported higher toxicity of propargite 57 EC against *T. urticae* with an LC_{50} value of 0.20 ppm and Kaur and Bhullar (2019) reported the lowest LC_{50} value of 0.002 ppm against the susceptible population of *T. urticae* to propargite 57 EC from Punjab. The increased susceptibility of the susceptible laboratory population might be due to increased generations in the laboratory without exposure to acaricides.

The LC_{50} value for spiromesifen was 0.098 ppm, agreeing with of Mohin et al. (2021). Based on the LC_{50} value, there was a significant difference between fenazaquin 10EC, propargite 57EC, and spiromesifen 22.9SC (0.070-0.100 ppm) with non-overlapping limits, and a non-significant difference was noticed for chlorfenapyr 10SC (0.130-0.180 ppm) with overlapping fiducial limits. Sridhar and Rani (2003) reported the susceptibility of *T. urticae* to dicofol in both open and greenhouse populations on roses with LC_{50} values of 0.040 and 0.019%, respectively. Similarly, the LC_{50} values of 0.97 ppm were reported abamectin (Patil et al., 2019). For chlorfenapyr 10SC against the red spider mite susceptible population was found to be the

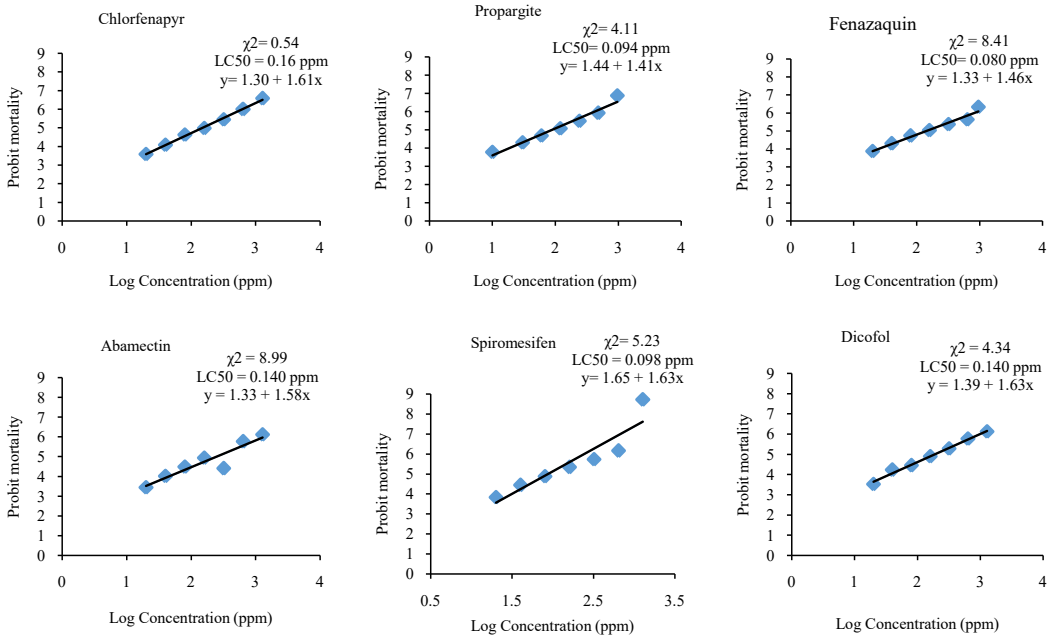


*Table χ^2 (df=5) value at 5% level of significance =11.07

Fig. 1. Regression lines showing the log concentration - probit mortality response of *T. urticae* to selected acaricides

least with an LC_{50} value of 0.160 ppm. A significant difference was noticed for fenazaquin 10EC, propargite 57EC, and spiromesifen 22.9SC (0.070-0.120 ppm) (Mohin et al., 2021) and 0.15 ppm was recorded against *T. urticae* (Naveena et al., 2019) reported the least susceptible value with the LC_{50} value of 0.42 and 0.15 ppm against *T. urticae* in Chikkamagaluru and Coimbatore region. Beers et al. (1998) concluded that the key factor driving acaricideresistance management is the availability of effective baseline susceptibility data. The establishment of baseline values (LC_{50} or LC_{90}) as a reference against the acaricide before its widespread use may be helpful for effective monitoring and understanding of the changes in its susceptibility over a period of time and can provide an opportunity to monitor the resistance before the instances of field failures. Baseline values estimated in the present study revealed susceptibility level of *T. urticae* when completely deprived of acaricide selection pressure.The susceptibility (LC50) varied from 0.08 to 0.16 ppm, which epitomizes two fold variation in toxicity between the six acaricides against the susceptible population.

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CONFLICT OF INTEREST

No conflict of interest.

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EFFICACY OF BIOPESTICIDES AND CHLORANTRANILIPROLE AGAINST TOMATO FRUIT BORER *HELICOVERPA ARMIGERA* (HUBNER)

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ABSTRACT

Field trial was conducted at the Central Research Farm, during rabi, 2022-2023. The experiment was laid out in RBD (randomized block design). Eight treatments were evaluated against *Helicoverpa armigera* (Hubner). Insecticides with treatment T_1 chlorantraniliprole 18.5%SC (1:10.) was the best. This was chlorantraniliprole 18.5%SC, ½ dose chlorantraniliprole + nisco sixer plus 2ml/ l (*O. sanctum* 13%+ *A. nardus* 4%), spinosad 45%SC, nisco sixer plus 2ml/ l (*O. sanctum* 13%+ *A. nardus* 4%) recorded the least fruit infestation. The highest yield was noticed in chlorantraniliprole 18.5%SC (240.5 q/ ha), followed by ½ dose chlorantraniliprole + nisco sixer plus 2ml/ l (*O. sanctum* 13%+ *A. nardus* 4%).

Key words: Chlorantraniliprole, cost benefit ratio, *Helicoverpa armigera,* incidence, spinosad, tomato, treatments, spinosad, NSKE, azadirachtin, nisco sixer plus, Ocimum, fruit infestation

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable ranks second among vegetables in area and production and occupies an area of 1.20 million ha. with a production of 19.4 mt with yield of 16.1 mt hectare. Among the various insect pests, tomato fruit borer *Helicoverpa armigera* is highly destructive causing serious damage. It has been found to cause a yield loss of 35 to 37.79% (Biswas et al., 2019). This study evaluates some biopesticides alongwith insecticides against the pest.

MATERIALS AND METHODS

The experiment was conducted under field conditions at the Central Research Farm, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh during rabi 2022-2023. Randomized block design with three replications was followed with plots 2× 1 m size maintaining 30 cm borders as a bunds and treatments was assigned randomly. The observations on infestation of *H. armigera* was recorded visually from five randomly selected tagged plants. The insecticides were sprayed at recommended doses when infestation reached ETL threshold. On seventh and fourteenth days after spraying observations were made on the number of fruits. Descriptive statistics was calculated using MS-EXCEL. ICAR WASP Statistics software was used.

RESULTS AND DISCUSSION

The insecticides viz., T_1 chlorantraniliprole 18.5%SC, T_2 ½ dose chlorantraniliprole + nisco sixer plus, T_3 Spinosad 45%SC, T_4 nisco sixer plus 2ml/ l, T_5 neem seed kernal extract, T_6 azadirachtin 5%, T_6 azadirachtin 5%, T_7 *Beauvaria bassiana* were evaluated against tomato fruit borer *H. armigera.* The spray was carried out during peak period of fruit borer and the data was recorded one day before treatment (DBT) and then on 7th and 14th day after treatment (DAT). The data on the efficacy of the treatments given in Table 1 revealed significant reduction in incidence after 7 and 14 days after treatment (DAT). Among all the treatments minimum percent infestation of fruit borer was recorded in T_1 chlorantraniliprole 18.5SC (8.57%) as compared to T_0 – untreated control (21.87%) similar to the findings reported by Jamir et al. (2022), Patil et al. (2018). Among all the treatments lowest number of fruit borer was recorded in chlorantraniliprole 18.5%SC. Second most effective treatment was recorded in T_2 ½ dose chlorantraniliprole + nisco sixer plus 2ml/ l (9.604%) these results were similar to the findings reported by Lalhluzuala and Kumar (2022). Third effective treatment was recorded in T_3 Spinosad 45%SC (10.66%), which are similar with Harshita et al. (2018). Fourth effective treatment was recorded in T_4 nisco sixer plus 2ml/ l (11.35%) these findings agree with those of Tejeswari et al., and Kumar (2021).

Higher yield (240.5 q/ ha) and higher cost: benefit ratio (1:10.4) was obtained from chlorantraniliprole 18.5%SC treated plots and lowest (110 q/ ha) in untreated control plot. These findings agree with

Table 1. Infestation of *H. armigera* in tomato

Sl. No.	Treatments	Fruit infestation (5 plants) in %							Mean	Yield (q/ ha)	C:B ratio
		First spray				Second spray					
		DBS	7DAS	14DAS	Mean	DBS	7DAS	14DAS	Mean		
T1	Chlorantraniliprole18.5%SC 1ml/ ℓ	21.67	8.44	10.59	9.515	11.85	6.64	8.62	7.63	8.57	240.5	1:10.4
T2	½Dose chlorantraniliprole + Nisco sixer plus 2 ml/ l 0.5 ml/ ℓ+ 2 ml/ ℓ	19.48	9.06	11.37	10.216	13.43	8.9	9.08	8.99	9.60	232.5	1:10.0
T3	Spinosad 45%SC 0.4 ml/ ℓ	18.69	10.25	12.05	11.15	14.43	9.63	10.0	9.81	10.66	200	1:8.7
T4	Nisco sixer plus 2 ml/ ℓ	18.91	11.23	13.07	12.15	14.87	10.52	10.59	10.56	11.35	187.5	1:8.1
T5	Neem seed kernel extract 5% 50 ml/ ℓ	18.31	15.00	13.86	14.43	15.32	11.29	11.60	11.44	12.93	160	1:6.9
T6	Azadirachtin 5% 5ml/ ℓ	18.02	16.31	15.31	15.81	16.86	12.80	12.50	12.65	14.23	150.5	1:6.5
T7	*Beauvaria bassiana* 2 g/ ℓ	20.36	17.66	16.17	16.92	17.51	14.39	13.46	13.92	15.42	135.5	1:5.8
T0	Control	21.53	23.19	25.33	24.26	23.14	18.86	19.49	19.17	21.87	110.5	1:4.9
	F-test	NS	S	S	S	S	S	S	S	S	-	-
	S. Ed. (±)	1.38	0.35	0.32	0.85	0.39	0.31	0.15	0.42	0.58	-	-
	C.D. (p=0.05)		1.047	0.995	2.842	1.175	0.941	0.463	1.396	1.954	-	-

DBS - Day Before Spray; DAS- Day After Spray; NS-Non significant; S- Significant

those of Jamir et al. (2022) who reported that the chlorantraniliprole 18.5%SC is the best and most economical treatment recorded yield (222.54q/ ha) and cost benefit ratio (1:9.14). Next highest yield and benefit cost ratio was recorded in T_2 –½ dose chlorantraniliprole + nisco sixer plus 2ml/ l (232.5 q/ ha and 1:10.0) similar findings made by Lalhluzuala and Kumar (2022). Bandhavi and Kumar (2019) who reported that the T_3 spinosad 45%SC is the best and most economical treatment which is similar to yield (200 q/ha) and cost benefit ratio (1:8.7). The results concluded that among all the treatments in chlorantraniliprole % SC with minimum mean 8.57% and maximum yield of 240.5q/ ha proved to be the best treatment which is followed by ½ chlorantranilirpole + nisco Sixer Plus 2ml/ l mean of 9.6% and yield with 232.5 q/ hac, and at last untreated control with mean of 21.87% and yield is 110.5q/ ha in managing *H. armigera* reduction. Recommended dose of chemicals may be useful in devising proper strategy against fruit borer of tomato.

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AUTHOR CONTRIBUTION STATEMENT

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No conflict of interest.

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FIRST REPORT OF INFESTATION OF THE AGAR DEFOLIATOR *HEORTIA VITESSOIDES* (MOORE) ON THE AGAR WOOD TREE *AQUILARIA MALACCENSIS* BENTH. IN SOUTH INDIA

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ABSTRACT

Massive infestation of the agar defoliator *Heortia vitessoides* (Moore) (Lepidoptera, Pyraloidea, Crambidae) was observed in Thiruvananthapuram and Kollam districts in Kerala on agar wood tree *Aquilaria malaccensis* Benth. (Thymelaeaceae), during February – December, 2022. Of the 119 trees observed in Thiruvananthapuram and Kollam districts, 115 were infested and six of them died due to the pest attack.

Key words: *Aquilaria malaccensis*, *Heortia vitessoides*, Thymelaeaceae, Crambidae, pest, south India, Kerala, damage, death of tree, defoliator, agar wood

Agar wood tree *Aquilaria malaccesnsis* Benth. (Thymelaeaceae), native to north east India, is known for the essential oil, extracted from the resinous hard wood formed in response to complex biotic, abiotic and physical stress (Naziz et al., 2019). The resinous hard wood of agar is the most expensive wood in the world as the price of agar wood oil is as much as US $ 50,000 to 80,000 per litre (Pearson, 2007). Assam in north east India is one of the globally important centres of agar wood cultivation and extraction of oil (Naziz et al., 2019). As the climate and soil in Kerala are more or less similar to those of Assam, the tree grows well in the state and there are many small and marginal farmers who have taken up the cultivation of agar wood expecting exorbitant returns. There is a network of such farmers spread across the state. Information on the insect pests of agar wood is limited. Samsuddin et al. (2019) reviewed the insect pests of agar wood plantations. The agar wood defoliator *Heortia vitessoides* (Moore, 1885) (Lepidoptera, Pyraloidea, Crambidae) is the most important pest of the tree (Figs. 1 - 5). The pest causes total defoliation of the trees. Consecutive cycles of defoliation, flushing and defoliation may cause death of the trees. Biology of *H. vitessoides* was studied by Singh et al. (2000) and Rahman (2018). Moore erected a new monotypic genus *Tyspana* for a new species, *T. vitessoides* from Sri Lanka (Moore, 1885). Hampson (1896) treated *Tyspana* as a synonym of *Heortia* Lederer, 1863. *Heortia vitessoides* is distributed in south-east Asia, including Bangladesh, Fiji, Hong Kong, India, Sri Lanka, Taiwan, Thailand and northern Queensland in Australia. In India, *H. vitessoides* has been reported in Arunachal Pradesh, Assam, Meghalaya, Nagaland, Sikkim, Tamil Nadu and Tripura. In south India, its presence has already been reported in Ponmudi in Thiruvananthapuram District and Asambu Reserve Forest in the neighbouring Kanyakumari District (Veino, 2022). Its host plants include *Aquilaria crassna* Pierre ex Lecomte, *Lagetta lagetto* Nash, *Phaleria lanceolata* Gilg (Robinson et

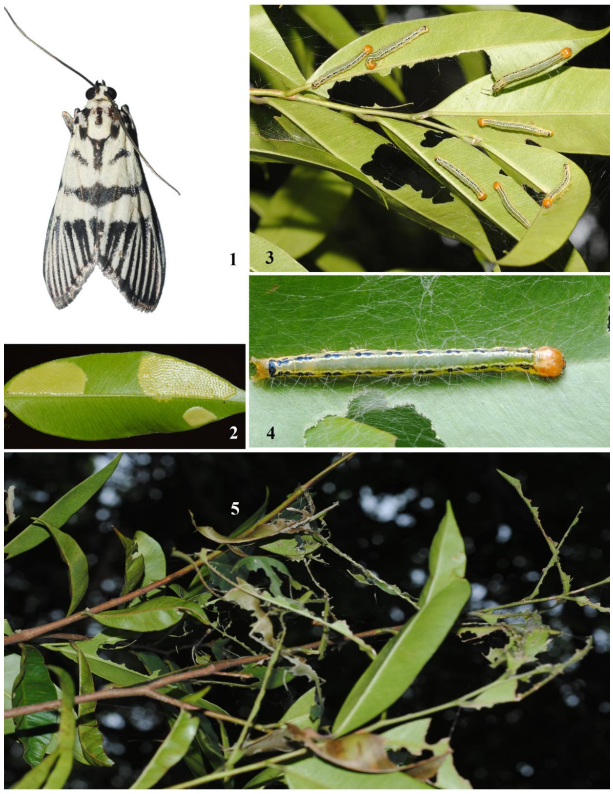


Fig. 1-5. *Heortia vitessoides*. 1. adult, 2. egg masses, 3. larvae and leaf damage, 4. larva, 5. damaged twig

Table 1. Extent of infestation of agar defoliator on agar wood tree in Thiruvananthapuram and Kollam districts, Kerala

Locality	GPS coordinates	No. of trees observed	No. of trees infested	No. of trees dead
Bonaccord, Thiruvananthapuram	Lat. 8.677699, Lng. 77.157892	1	1	0
Channapetta, Kollam	Lat.8.8856372, Lng. 76.9655662	20	20	0
Chavara, Kollam	Lat. 8.9958117, Lng. 76.5451889	75	75	3
Edava, Kollam	Lat. 8.7725190, Lng. 76.6848140	11	11	3
Pulimath, Thiruvananthapuram	Lat. 8.737004, Lng. 76.874366	10	6	0
Thonnakkal, Thiruvananthapuram	Lat. 8.633435, Lng. 76.859460	2	2	0

al., 2010), *Aquilaria malaccensis* (Singh et al., 2000) (all Thymelaeaceae) and *Rhus* L. sp. (Anacardiaceae) (Herbison-Evans & Crossley, 2010). Law et al. (2022) presented a chromosomal-level assembly for *H. vitessoides*, consisting of a 517 megabase (Mb) genome assembly. Qian et al. (2023) showed that *Metarhizium anisopliae* (Metschn.) Sorokin, can effectively kill the gregarious larvae of *H. vitessoides* through direct contact and horizontal transmission.

MATERIALS AND METHODS

Massive infestation of the agar defoliator was observed in Thiruvananthapuram and Kollam districts in Kerala on agar wood tree during February – December, 2022. Field visits were carried out to assess the nature and extent of damage. A voucher specimen of *H. vitessoides* (Accession number: NIM/NBAIR/LEP/HEOR/30522) is deposited in the ICAR-National Bureau of Agricultural Insect Resources, Bengaluru.

RESULTS AND DISCUSSION

Infestation was recorded at Bonaccord, Pulimath and Thonnakkal in Thiruvananthapuram district, and Channapetta, Chavara and Edava in Kollam district (Table 1; Fig. 1-5). All the trees examined were affected, except four out of 10 in Pulimath. In Chavara, of the nearly 75 trees, three died due to the infestation, while in Edava three trees out of 11 were lost. Eggs were observed in masses (Fig. 2) on the abaxial surface of tender leaves. The larvae (Figs. 3, 4) webbed the leaves together and fed on the leaves (Fig. 5). In most cases, defoliation was complete and the tender branches dried up. This is the first report of infestation of *H. vitessoides* on *A. malaccensis* in south India. As the cultivation of agar wood tree is gaining popularity in Kerala, there is an urgent need to develop sustainable management measures, including cultural, biological and chemical methods.

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AUTHOR CONTRIBUTION STATEMENT

PKD conceptualized the study, identified the pest, carried out field work and prepared the first draft of the manuscript. TS carried out field work, survey and revision of the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF REARING METHODS ON *SPODOPTERA LITURA* (F) UNDER LABORATORY CONDITION

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Effects of natural and artificial diets under two rearing methods (group and individual) on larval and pupal development and survivability of *Spodoptera litura* (Fab.) were studied. The survival rate of larvae and pupae was significantly affected by diets (P = 0.05), which was higher on artificial diet when reared individually. Results of this study documented the higher larval and pupal growth index from artificial diet. The larval growth indices were 5.24 and 1.65 on artificial diet under individual group rearing, respectively, and 2.35 on natural diet under group rearing. The developmental periods of larvae and pupae were not significantly affected by diets, which were shorter on artificial diet reared in group.

Key words: *Spodoptera litura*, artificial diet, rearing methods, adult emergence, reproductive competence, larval growth index, pupal growth index, larval survival, pupal survival

Spodoptera litura (Fab.) is a polyphagous species widely distributed throughout the world due to its migratory behavior (Rani and Mohan, 2000; Narayanan, 2003). Around 112 plant species belonging to 44 families have been reported as its food plants (Xue et al., 2010). The pest is capable of eating leaf, flowers, fruit etc. and 26 to 100% losses have been documented depending upon the crop stage and its infestation level in the field (Daniel and Samiayyan, 2017). Rearing of an insect species under artificially controlled conditions is the pre-requisite for its continuous supply, for conducting various experiments regarding evaluation of toxicity and efficacy of insecticides. Diet and rearing methodology plays a critical role on the growth and development, survival, adult behavior, and reproductive competence of reared insect. Literature revealed that different workers used different rearing regimes for larval rearing of *S. litura* like rearing larvae in group on natural diet or artificial diet and rearing individual larvae in separate vials, but, the studies on comparison of different rearing regimes of *S. litura* are lacking. Therefore, present study has been conducted to compare the effects of rearing methods on growth index and survival and survivability of *S. litura*.

MATERIALS AND METHODS

The present investigation was carried out at ICAR-NRCIPM and Division of Entomology, ICAR- IARI, Pusa Campus, New Delhi. The experiment was conducted in a growth chamber (Accumax India) during 2018-19. Fourth and fifth instar larvae of *S. litura* were collected from the fields of ICAR-IARI, in 2018, and reared on fresh castor leaves. The culture was maintained at 27± 1ºC and 65± 5 % RH with a 14:10 L: D photoperiod. The experiment was conducted with four treatments i.e. artificial diet- individual (ADI) artificial diet in group (ADG) group rearing on natural diet i.e. castor leaf (NDG) (N.D.G) and individual rearing on natural diet (NDI).

Group and separate larval rearing on natural diet was done by providing fresh castor leaves. Neonates (<12 hrs) were transferred to sterilized glass container (22 cm x 14 cm) which had a small cup half-filled with tap water where petioles were submerged to prevent the desiccation of leaf and about 30-40 neonates were released in each of the two leaves maintained per container. Fresh castor leaves were sterilized by 1% sodium hypochlorite solution followed by washing with tap water about 3-4 times before feeding in every alternate day. After 5th day of hatching in group rearing, 10 larvae were transferred to each propylene jar (20 cm x 15.5 cm) whereas for separate rearing the larvae were individually placed in small rectangular vials (5 cm x 2.5 cm x 4.5 cm). Survivability was recorded at every 24 hr until the emergence of healthy adult. Pre-pupae were allowed to pupate inside jar till completion of sclerotization and pupae were transferred to new sterilized petri plates for adult emergence.

Group and individual larval rearing on artificial diet was done by providing chickpea flour based diet, prepared as per the protocol reported by Gupta et al.,

2005[a] with some modifications. Neonates were reared in the same manner as described above on castor leaves. After 5th day of hatching, the survived larvae were individually placed in small rectangular vials for individual rearing and 10 larvae for group rearing were placed in a Petri plate (16 x 3 cm). Larvae were allowed to pupate inside the artificial diet till 48 hr and shifted from diet to vials for adult emergence.

Observations were recorded on some attributes of growth and development of the immature stages of *S. litura*. Since NDI resulted inconsistent feeding by larvae and malformation in pupae, poor quality of malformed pupae, it was excluded from data analysis of growth indices. One day old neonates were used to measure the biological characteristics, selected randomly from the same egg patch, larvae were weighed on 8th, 14th and 17th day of hatching, respectively which was standardized as optimal time to measure the diet accessibility and growth (Sridhar et al., 2013). Only healthy, live pupae were weighed and counted. The larvae with unsuccessful moulting and adult with malformed organs were considered dead. The larval and pupal growth indices were calculated using the following formulae (Gupta et al., 2005[b]): Larval growth index= % pupation/ larval duration (days); Pupal growth index= % of emerged moths/ pupal duration (days); Larval period (days), larval survivability (%), larval weight (mg), pupal period (days), pupal weight (mg), rate of pupation (%) and rate of adult emergence (%) were recorded. Each treatment was replicated three times and ten larvae per replication. The experiment was laid out in completely randomized design with 4 treatments and 3 replications; data analysis was done by using WASP 2.0 package.

RESULTS AND DISCUSSION

Mean percentage of survival of 1st and 2nd instar larvae of *S. litura* were at par in all the treatments (Table 1). Survivability of 3rd instar larvae was significantly higher in ADI (98.05%) followed by ADG and NDG. Similarly survival of 4th, 5th and 6th instar larvae in ADI was significantly higher followed by NDG and ADG. Consequently pre-pupation rate was also observed highest on ADS (93.61%) followed by NDG (51.12%) and lowest on ADG (38.47%). The duration of 1st, 2nd and 4th instar larvae and prepupal stage was statistically at par in all the treatments. Duration of 6th instar was lowest in ADI (1.15 days) followed by ADG and NDG. Total larval period was minimum in ADI (16.68 days) followed by ADG and NDG. The mean larval weight was significantly higher in ADI. Pupation was significantly higher on ADI (89.44%), followed by NDG and ADG. The pupal periods were at par in all treatments. Mean pupal weight was significantly affected by different treatments which was maximum in ADI (323 mg) followed by ADG and NDG. Rate of adult emergence was also highest in ADI (86.8%) followed by NDG and ADG. ADI recorded the highest larval growth index (5.24) and pupal growth index (11.42) followed by NDG and ADG (Table 1).

According to Bernays and Chapman (1994) growth, development, survival and reproduction in phytophagous insects significantly affected by the chemical composition of diet and development of larvae is influenced by the food quality. In this study, we have examined the effect of different diets on larvae development. Although, a long list of different artificial diets have been reported for the culturing and maintenance of the such economically important insect pest, generally for lepidopteran larvae (Cohen, 2001) but there is always a chance of the refinement of existing standard protocols. The lowest mean survival rate of larvae and pupae on natural food indicates that castor leaves failed to supply required nutrients which were essential to change the consumption rate into different metabolic reactions. Chickpea was the main source of carbohydrate (Seth and Sharma, 2002), to avoid the chances of fungal and other secondary infections, microbial inhibitors were mixed during the preparation of diet for example streptomycin, sorbic acid, methyl-p-hydroxybenzoate (methyl paraben). L-ascorbic acid used as indirect source of vitamin-c, which is an essential requirement for normal growth of herbivores. The study showed low survival of *S. litura* larvae under group rearing condition on artificial diet and natural diet in comparison to separate rearing on artificial diet, which may be due to the cannibalism among the larvae because of limited space and competition for food. According to Bloom et al. (2010) insects were very sensitive to any type of stress (ecological and biological) to their surroundings, that can totally change their growth and development. Previous studies reported that non motile (egg and pupae) or less motile stages (younger larvae) are more prone to cannibalism by older larvae and more on natural host when compare to artificial diet in mass rearing. Ryuda et al. (2008), favored to rear larvae separately on artificial diet to avoid cannibalism in tobacco cutworm. Separate or singly rearing on artificial diet in vials, is a good option to avoid cannibalism in laboratory rearing. Janakiraman and Gupta (2002) reported 86.2% pupation, 325.9mg

Table 1. Survival, growth and development of *S. litura* on artificial diet and castor leaf

Treatment	Different stages of *S. litura*					
	3rd Instar	4th Instar	5th Instar	6th Instar	Pre- pupa	Pupa
ADI	98.05 (85.14)[a]	97.23 (83.10)[a]	96.39 (81.06)[a]	94.16 (77.67)[a]	93.61 (77.01)[a]	89.44 (72.28)[a]
ADG	86.69 (66.43)[b]	73.2 (59.79)[c]	58.05 (49.79)[c]	48.89 (44.33)[c]	38.47 (38.17)[c]	29.16 (32.50)[c]
NDG	85.55 (68.22)[b]	78.33 (62.72)[b]	73.33 (59.38)[b]	60.03 (50.56)[b]	51.12 (45.63)[b]	37.5 (37.62)[b]
S.Em	0.087	0.076	0.08	0.105	0.055	0.1
CD (p=0.05)	7.89	7.94	5.91	5.05	5.44	5
Treatment	3rd Instar	4th Instar	5th Instar	6th Instar	Pre- pupa	Pupa
ADI	3.54 (1.91)[a]	4.075 (2.019)[a]	2.37 (1.82)[a]	1.15 (1.59)[a]	1.02 (1.24)[a]	7.6 (2.67)[a]
ADG	3.13 (1.8)[a]	4.04 (2.061)[a]	2.32 (1.69)[a]	1.38 (1.67)[a]	1.44 (1.37)[a]	7.3 (2.87)[a]
NDG	2.78 (0.707)[b]	4.42 (1.761)[b]	2.2 (1.52)[a]	2.47 (0.707)[b]	1.47 (1.17)[a]	8.28 (3.05)[b]
S.Em	0.080	0.059	0.084	0.066	0.048	0.103
CD (p=0.05)	0.11	0.167	NS	0.11	NS	0.197
CV	3.26	3.42	5.41	11.93	10.56	3.94

Growth and development (Mean± S.Em)

Parameter	T1	T2	T3
Larval weight (mg) (8-day-old)	50.06± 1.23	49.58± 1.85	48.17± 2.6
Larval weight (mg) (14-day-old)	428.04± 34.07	312.64± 27.74	122.18± 10.01
Larval weight (mg) (17-day-old)	742.15± 63.45	608.76± 4.70	279.44± 61.25
Larval period (days)	17.06± 0.49	17.88± 0.55	15.9± 0.35
Pupal period (days)	7.6± 0.15	7.3± 0.19	8.28± 0.18
Pupal weight (mg)	323± 19.77	249± 11.29	190.52± 26.92
% of Emerged Adults	86.8± 0.57	28.01± 4.34	34.13± 3.66
Larval growth Index	5.24	1.65	2.35
Pupal growth Index	11.42	3.83	4.12

ADI artificial diet; ADG: artificial diet group; NDG: group rearing natural host; *Figure in parenthesis are arc sign transformed values; values superscript with same letter are statistically *at par*. Artificial diet-individual; ADG: artificial diet group; NDG: group rearing on natural host; Figures in parenthesis are square root transformed values and values superscript with same letter are statistically *at par*.

pupal weight, 92.3% adult emergence and 83.8% survival rate, for the same insect when reared on artificial diet. Narvekar et al. (2018) reported 7.33 days pupal period on castor, however, it was found to be longer (8.28 days) on castor in our study. Our results on artificial diet in separate rearing indicating highest larval and pupal growth index which seem to be co-incide with the results by Divakara and Manjulakumari, (2015). It can be concluded that the group rearing of *S. litura* whether on artificial or natural host (castor leaf) is not feasible in the laboratory conditions. Individual rearing on artificial diet is a good alternative to group rearing on natural/ artificial diet.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

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SCREENING OF MUNGBEAN GENOTYPES AGAINST WHITEFLY *BEMISIA TABACI* (GENN.) AND THRIPS *MEGALUROTHRIPS DISTALIS* (KARNY)

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ABSTRACT

Ten green gram genotypes (K 851, GM 4, Meha, VMS 6 (GM-5), IPM 205-7 (Virat), Vaibhav, AKM-6802, CO-5, AKM-8803 and PM-2) were screened for their resistance to the sucking pests. The field experiment was conducted at the Instructional Farm, JAU, Junagadh during summer 2018. None of the varieties proved highly resistant to the whitefly *Bemisia tabaci* (Genn.) and thrips *Megalurothrips distalis* (Karny); however, Meha, IPM 205-7 (Virat), and VMS 6 (GM-5) were found resistant. The varieties Vaibhav, CO-5 and PM-2 have been classified as moderately resistant (MR) to *B. tabaci* and AKM- 6802 was moderately susceptible (MS) to both the pests. The susceptible (S) category included the AKM-8803, GM-4, and K-851 varieties.

Key words: Genotypes, summer, green gram, screening, sucking pests, *Megalurothrips distalis*, *Bemisia tabaci,* resistant, susceptible, population, infestation

Green gram *Vigna radiata* (L.) Wilczek is grown primarily for its grains, particularly in Asia and Africa (Nair et al., 2013), and it is grown in tropical and subtropical regions. Globally, the production of green gram is limited by incidence of diseases, pest infestations, use of unsuitable varieties and adaptation of inappropriate agronomic practices (Rao et al., 2000), and insect pests are the major ones (Davies and Lateef, 1975; Saxena, 1978; Seif et al., 2001) accounting for 54.9% loss (Chhabra and Kooner, 1998). The whitefly *Bemisia tabaci* (Gennadius) has been a significant sucking pest, and also by spreading the yellow mosaic viral disease (Taggar and Gill, 2016). The avoidable losses due to *B. tabaci* have been reported to range from 25 to 78% (Sharma and Verma, 1984). Thrips *Megalurothrips distalis* (Karny) is a major sucking pest in green gram (Chhabra and Kooner, 1998). Thrips attack the crop at the flowering stage, causing flower deformity and sometimes leads crop failure. Thrips not only cause direct damage by feeding, but also serve as a vector for various plant viruses (Ananthakrishnan, 1980). Different strategies, mainly with insecticides have been recommended against such sucking insect pests, but many times these are ineffective, necessitating development of alternative tactics. Host plant resistance (HPR) and use of natural enemies, including predators and parasitoids are such ecofriendly measures. Host plant resistance offers solution for maintaining whitefly and thrips populations and reducing crop losses (Bellotti and Arias 2001; Mouden and Leiss, 2020). Identifying suitable resistant varieties under field conditions is required for this, and in the present study, some green gram genotypes have been screened for resistance to *B. tabaci* and *M. distalis*.

MATERIALS AND METHODS

The experiment was conducted at Instructional Farm, Department of Agronomy, COA, JAU, Junagadh during summer 2018. The recommended agronomic practices were followed, with the seeds sown in a randomized block design (RBD) plots (4.00x 0.90 m^2) with ten treatments and three replications; the genotypes evaluated include- K 851, GM 4, Meha, VMS 6 (GM-5), IPM 205-7 (Virat), Vaibhav, AKM-6802, CO-5, AKM-8803 and PM-2 usually grown in the Junagadh region. The varieties were allowed natural infestation, with incidence of pests recorded from ten randomly selected plants. Mature and immature stages were counted at 15-day intervals from the first week after sowing until crop maturity. Incidence of *B. tabaci* and *M. distalis* was counted from three leaves representing each from top, middle and bottom canopy of each plant. These counts were subjected to square root transformation before statistical analysis. The genotypes were categorized into six groups of resistance as: highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible (Kansagara, 2017).

The mean value of each variety ($\overline{X}_i$) was compared to the mean value of all varieties ($\overline{X}$) and the standard deviation was calculated (SD). The retransformed data was used to compute $\overline{X}$, $\overline{X}_i$ and SD.

RESULTS AND DISCUSSION

The data on the incidence of *B. tabaci* revealed that all the genotypes were infested, of which Meha recorded the least counts (1.44/ leaf), which was comparable to IPM 205-7 (Virat; 1.48/ leaf) and VMS-6 (GM-5; 1.53/ leaf), which can be considered as the least susceptible; maximum counts were observed with the genotype K-851 (3.88 /leaf), which in turn was at par with GM-4 (3.83/ leaf) and AKM-8803 (3.76/ leaf) which can be considered as highly susceptible. None of the genotypes was highly resistant (HR) to *B. tabaci;* Meha, IPM 205-7 and VMS 6 (GM-5) were found resistant (R); Vaibhav, PM-2, and CO-5 were observed to be moderately resistant (MR). None of the varieties was found highly susceptible (HS) (Table 1). Kingsly *et al.* (2015) reported the resistance level of genotypes to *B. tabaci* and identified C0-5 and K-851 as the highly susceptible. Suman *et al.* (2015) documented Meha, PM-2 and AKM-8803 as resistant, moderately susceptible and susceptible, while Mohan *et al.* (2014) amongst 120 germplasm against MYMV and reported ten genotypes including IPM 205-7 as resistant, and 46 genotypes as susceptible.

As regards *M. distalis* none of the genotypes were free of infestation, with a minimum of 1.01 thrips/ leaves being with IPM 205-7 (Virat) and which was at par with Meha (1.04 thrips/ leaves) and VMS-6 (GM-

Table 1. Resistance to *B. tabaci* and *M. distalis* in green gram

Category of resistance	Scale	Genotypes $\overline{X}_i$	
1	2	3	
	Based on three leaves upper, middle and lower (whitefly /leaf): $\overline{X}$ = 2.54 and SD = 0.99		
B. tabaci			
Highly resistant	$\overline{X}_i$ < (0.56)	-	
Resistant	$\overline{X}_i$ > (0.56) < (1.55)	Meha	(1.44)
		IPM 205-7	(1.48)
		VMS 6	(1.53)
Moderately resistant	$\overline{X}_i$ > (1.55) < (2.54)	Vaibhav	(2.05)
		PM- 2	(2.24)
		CO-5	(2.31)
Moderately susceptible	$\overline{X}_i$ > (2.54) < (3.53)	AKM- 6802	(2.87)
Susceptible	$\overline{X}_i$ > (3.53) < (4.52)	AKM- 8803	(3.76)
		GM- 4	(3.83)
		K- 851	(3.88)
Highly susceptible	$\overline{X}_i$ > (4.52)	-	
M. distalis			
Highly resistant	$\overline{X}_i$ < (0.03)	-	
Resistant	$\overline{X}_i$ > (0.03) < (1.16)	IPM 205-7	(1.01)
		Meha	(1.04)
		VMS 6	(1.11)
Moderately resistant	$\overline{X}_i$ > (1.16) < (2.29)	Vaibhav	(1.49)
		PM- 2	(2.02)
Moderately susceptible	$\overline{X}_i$ > (2.29) < (3.42)	CO-5	(2.32)
		AKM- 6802	(2.82)
Susceptible	$\overline{X}_i$ > (3.42) < (4.55)	AKM- 8803	(3.59)
		GM- 4	(3.71)
		K- 851	(3.78)
Highly susceptible	$\overline{X}_i$ > (4.55)	-	

(contd.)

Categorization

Category	Scale of resistance
Highly resistant	$\overline{X}_i < (\overline{X} - 2SD)$
Resistant	$\overline{X}_i > (\overline{X} - 2SD) < (\overline{X} - SD)$
Moderately resistant	$\overline{X}_i > (\overline{X} - SD) < \overline{X}$
Moderately susceptible	$\overline{X}_i > \overline{X} < (\overline{X} + SD)$
Susceptible	$\overline{X}_i > (\overline{X} + SD) < (\overline{X} + 2SD)$
Highly susceptible	$\overline{X}_i > (\overline{X} + 2SD)$

Incidence

S. No.	Genotypes	No. of insect pests/leaf/plant[&]	
		Whitefly	Thrips
1	K 851	1.97* (3.88)	1.94 (3.78)
2	GM 4	1.96 (3.83)	1.92 (3.71)
3	Meha	1.20 (1.44)	1.02 (1.04)
4	VMS 6 (GM-5)	1.24 (1.53)	1.05 (1.11)
5	IPM 205-7 (Virat)	1.22 (1.48)	1.01 (1.01)
6	Vaibhav	1.43 (2.05)	1.22 (1.49)
7	AKM-6802	1.69 (2.87)	1.68 (2.82)
8	CO-5	1.52 (2.31)	1.52 (2.32)
9	AKM-8803	1.94 (3.76)	1.90 (3.59)
10	PM-2	1.50 (2.24)	1.42 (2.02)
	S.Em.±	0.07	0.07
	CD (p=0.05)	0.21	0.21
	CV %	8.51	8.70

Figures in parentheses whitefly /leaf; $\overline{X}_i$ = Mean value of individual variety; $\overline{X}$ = Mean value of infestation of all varieties; SD= Standard deviation; *Square root transformed values; [&]Figures in parentheses retransformed values

5) (1.11 thrips/ leaves); K-851 harboured a maximum incidence (3.78 thrips/ leaves), which was at par with GM-4 (3.71 thrips/ leaves); the rest of the varieties, Vaibhav, PM-2, CO-5, AKM-6802 and AKM-8803 can be considered as moderately susceptible; thus, IPM 205-7, Meha, and VMS-6 were found comparatively resistant. None of the genotypes could be classified as highly resistant (HR)- IPM 205-7, Meha and VMS 6 were all found resistant (R); Vaibhav and PM-2 were moderately resistant (MR), while CO-5 and AKM-6802 were moderately susceptible (MS); and AKM-8803, GM-4 and K-851 were classified as susceptible (S) category, with none as highly susceptible (HS) group (Table 1). Similar conclusions were drawn by Ghose and Chatterjee (2016), while Kansagara (2017) observed IPM 205-7 (Virat) as least susceptible, and K-851 and GM-4 as susceptible.

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AUTHOR CONTRIBUTION STATEMENT

GRH and BBK conceived and designed research. GRH and BBK conducted experiments. GRH and MCK analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFICACY OF INSECTICIDES AGAINST GRAM POD BORER *HELICOVERPA ARMIGERA* ON SOYBEAN

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ABSTRACT

The effect of various insecticides on the larval incidence of pod borer *Helicoverpa armigera* in soybean revealed that indoxacarb 14.5SC (69.44%) followed by imidacloprid 17.8SL (62.65%), cypermethrin 10EC (58.06%), profenophos 50EC (54.05%), quinalphos 25EC (48.83%) and neem oil (40.59%) led to maximum reduction. Their effect on the yield of soybean revealed that the maximum yield was with indoxacarb 14.5EC (19.19) followed by others.

Key words: Soybean, *Helicoverpa armigera*, indoxacarb, incidence, larva, imidacloprid, cypermethrin, profenophos, quinalphos, neem oil, yield

Soybean *Glycine max* (L.) is cultivated in 120.50 million ha with a productivity of 2785 kg/ ha (Anonymous, 2020a). In India, its productivity is 1005 kg/ ha (Anonymous, 2020b). Like other crops, soybean is also attacked by numerous pests. *Helicoverpa armigera* (Hubner) causes serious damage in the vegetative stage of crop as they mostly target axillary buds of the plants which give rise to the floral buds. This pest damage results in leaf chewing, terminal damage and damage to pods. Vegetative soybeans are more prone to *H. armigera* than that of other pulses. The present study evaluates the efficacy of some insecticides on the incidence of *H. armigera* in soybean.

MATERIALS AND METHODS

The field experiment was carried out in the experimental field of Division of Entomology, Faculty of Agriculture, Wadura, during kharif 2020. Soybean crop was grown adopting recommended SKUAST-Kashmir package of practices. The experiment was laid out in randomized block design (RBD) with three replications in plots of size 2 m^2 each. The crop was sown in the 3rd week of May, 2020 with row to row and plant to plant spacing of 45x 5 cm. There were seven treatments along with an untreated check. The insecticides viz., cypermethrin 10EC (0.006%), indoxacarb 14.5EC (0.007%), profenophos 50EC (0.05%), neem oil (0.3%), imidacloprid 17.8SL (0.005%), quinalphos 25EC (0.025%) along with control (water spray) were evaluated as foliar sprays with knapsack sprayer with spray fluid of 500 l/ ha. Pretreatment observations on larval incidence was recorded a day before spraying, while post treatment observations were taken after 1st, 3rd, 7th, 10th day after treatment (DAT) from 5 randomly selected and tagged plants. The % reduction of incidence over pretreatment was worked out following Abbot's (1925) formula: The data obtained on % reduction were analyzed statistically using ANOVA (p=0.05)

RESULTS AND DISCUSSION

The incidence of *H. armigera* ranged from 30.16-85.05%, 37.90-95.83%, 28.24-80.09%, 21.75-55.71%, 27.38-91.07% and 26.19-63.49% after treatment of chemical pesticides, viz., cypermethrin 10EC (0.006%), indoxacarb 14.5EC (0.007%), profenophos 50EC (0.05%), neem oil (0.3%), imidacloprid 17.8 SL (0.005%) and quinalphos 25 EC (0.025) in comparison to control (4.17-13.09%), respectively (Table 1). The mean % reduction was found to be maximum with indoxacarb 14.5EC 0.007% as 37.90, 57.94, 86.11 and 95.83% at 0.5 ml/ l. With pooled data cypermethrin 10EC (0.006%), indoxacarb 14.5EC (0.007%), profenophos 50EC (0.05%), neem oil (0.3%), imidacloprid 17.8 SL (0.005%) and quinalphos 25 EC (0.025) revealed reduction of 58.06, 69.44, 54.05, 40.59, 62.65 and 48.83%, respectively. Yogeshwardu

Table 1. Efficacy of insecticides against pod borer *H. armigera* in soybean

Treatment	Concentration (%)	Dosage (ml/ l)	Pretreatment count (1 DBT)	Reduction of larvae over pre-treatment (%)				Cumulative mean (%)
				1st	3rd	7th	10th	
Cypermethrin 10EC	0.006	0.6	6.67 (2.58)	30.16 (33.29)	50.00 (45.00)	69.84 (57.21)	85.05 (71.28)	58.06 (51.69)
Indoxacarb 14.5EC	0.05	0.5	7.00 (2.64)	37.90 (37.97)	57.94 (49.61)	86.11 (71.97)	95.83 (83.09)	69.44 (60.67)
Profenophos 50EC	0.05	1.0	8.33 (2.88)	28.24 (31.96)	48.16 (43.94)	68.06 (55.66)	80.09 (63.72)	54.05 (48.82)
Neem oil	0.3	3.0	6.00 (2.44)	21.75 (27.66)	33.97 (35.60)	50.95 (45.55)	55.71 (48.29)	40.59 (39.27)
Imidacloprid 17.8SL	0.005	0.5	7.33 (2.71)	27.38 (31.54)	54.76 (47.74)	77.38 (61.82)	91.07 (75.69)	62.65 (54.20)
Quinolfos 25EC	0.025	1.0	6.33 (2.51)	26.19 (30.56)	47.62 (43.63)	57.94 (49.61)	63.49 (52.86)	48.83 (44.16)
Water			7.67 (2.77)	13.09 (21.20)	12.50 (16.90)	08.33 (13.80)	04.17 (6.90)	9.52 (14.70)
C.D. (P≤ 0.05)			1.21 (0.23)	9.94 (6.64)	9.58 (10.34)	15.50 (14.83)	12.59 (15.07)	

*Mean of three replications; Figures in parentheses arc sine transformed values; DAT = Days after treatment; DBT: Days before treatment

et al. (2014) observed that indoxacarb 14.5 SC (1 ml/ l) was the most effective against H. *armigera.* Singh et al. (2012) also observed similar results. Kumar et al. (2013) evaluated the efficacy of some insecticides against *H. armigera* in soybean, and observed that spinosad 0.025%, thiodicarb 0.15%, and indoxacarb 0.0029% were the most effective against *S. litura* and rynaxypyr 0.006% against *H. armigera*. All the treatments significantly increased the yield of soybean with indoxacarb 14.5EC giving maximum (17.08 q ha^{-1}) followed by imidacloprid 17.8 SL and others, thus giving 6.98 to 19.19% increase in yield over control. Gowda et al. (2006) observed that spinosad 35SC and indoxacarb 14.5SC are the most effective in increasing grain yield. Singh et al. (2012) found indoxacarb treated plots giving maximum grain yield.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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EVALUATION OF IMIDACLOPRID 6%+ LAMBDA CYHALOTHRIN 4% SL AGAINST SUCKING PESTS OF COTTON

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ABSTRACT

Two field experiments were conducted to evaluate the efficacy of imidacloprid 6%+ lambda cyhalothrin 4% SL against sucking insects viz., aphid *Aphis gossypii* Glover, leafhopper *Amrasca biguttula biguttula* (Ishida), thrips *Thrips tabaci* L. and whitefly *Bemisia tabaci* (Gennadius) on cotton during kharif, 2018-19 and 2019-20 at the Agricultural Research Station, Dharwad farm, UAS, Dharwad, Karnataka. Results showed that the higher dose of imidacloprid 6%+ lambda cyhalothrin 4%SL @ 500 ml ha^{-1} resulted in 89.02, 88.52, 91.91 and 90.17% reduction of aphids, thrips, leafhoppers and whitefly, respectively in first season and 93.03, 87.15, 97.01 and 89.65% in second season compared to the untreated check. It was on par with its lower dose @ 450 ml ha^{-1}. There was no significant difference in the counts of grubs and adults of coccinellids and number of chrysopids. Thus, imidacloprid 6%+ lambda cyhalothrin 4% SL could be used for the management of sucking pests without any effect on non-target insects.

Key words: Cotton, *Aphis gossypii*, *Amrasca biguttula biguttula*, *Thrips tabaci*, *Bemisia tabaci*, imidacloprid, lamda cyhalothrin, natural enemies, coccinellids, chrysopids, yield

Cotton (*Gossypium hirsutum* L.) is an important commercial fibre crop, and India is one of the major producers (ICAC, 2021). It is estimated that >5.8 million farmers cultivate cotton in India and about 40-50 million people are employed directly or indirectly by the cotton industry (Karthik et al., 2017). Nearly 1326 insects and mites are reported on cotton (Hargreaves, 1948) and about 251 arthropods from India (Nagrare et al., 2022), with the yield loss of 87% (Taley et al., 1988). Introduction of Bt cotton has given solution to the bollworm complex to a larger extent but at the same time they are susceptible to most of the sucking pests viz*.,* aphid, leafhopper, thrips, whitefly and mirid bug. Cotton growers in India depend heavily on synthetic pesticides to combat these pests; at least 6-9 sprays are directed against sucking pests at early stage (Lingappa et al., 2001) and it has been estimated that cotton consumes about 21% of the total insecticides used in the country (CICR, 2011). Due to the continuous and indiscriminate use of these systemic insecticides, their efficacy is lost due to buildup of resistance to these insecticides. Hence, combination of two chemicals with different mode of action is the new strategy to reduce the labour, money, time and development of resistance. With this background, this study evaluates the efficacy of new combination product imidacloprid 6%+ lambda cyhalothrin 4% SL against sucking pests of cotton and its effect on natural enemies.

MATERIALS AND METHODS

Field trials were conducted to evaluate the efficacy of imidacloprid 6%+ lambda cyhalothrin 4% SL at the Agricultural Research Station, Dharwad farm, Dharwad during kharif, 2018-19 and 2019-20 in randomized block design (RBD). There were nine treatments replicated thrice. Cotton hybrid 'First class' was sown in plots of 5.0 x 5.0 sq. m with a spacing of 90 x 60 cm, and the crop was raised following recommended package of practices. Spraying was carried out using hand operated pneumatic knapsack sprayer with 500 l of spray fluid/ ha when the pests crossed economic threshold level (ETL) during 60 DAS (days after sowing) and untreated check plots were maintained with water spray. Three sprays were given at 15 days interval. The incidence of sucking pests viz*.,* nymphs and adults of aphids *Aphis gossypii* Glover, leafhopper *Amrasca biguttula biguttula* (Ishida), thrips *Thrips tabaci* L. and whitefly *Bemisia tabaci* (Gennadius) were recorded from ten randomly selected and tagged plants/ replication. In each plant, three leaves (top, middle and bottom) were considered, and observations were made prior to and 3, 7 and 14 days after spraying. Cotton

yield/ plot was recorded from each picking and pooled to arrive at the total yield in q/ ha. The occurrence of natural enemies (chrysopids and coccinellids) was recorded on ten randomly tagged plants/ plot prior to and 3, 7, 10 and 14 days after spray. Based on these observations, means were worked out and statistically analyzed after square root transformation. To assess the phytotoxicity, imidacloprid 6%+ lambda cyhalothrin 4% SL at four doses viz., 400, 450, 900 and 1350 ml ha^{-1} were used. The treatments evaluated were imidacloprid 6%+ lambda cyhalothrin 4% SL@ 350 ml ha^{-1}, imidacloprid 6%+ lambda cyhalothrin 4% SL@ 400 ml ha^{-1}, imidacloprid 6%+ lambda cyhalothrin 4% SL@ 450 ml ha^{-1}, imidacloprid 6%+ lambda cyhalothrin 4% SL@ 500 ml ha^{-1}, lambda cyhalothrin 5% EC @ 500 ml ha^{-1}, imidacloprid 70% WG @ 35 g ha^{-1}, buprofezin 25% SC @1000 ml ha^{-1}, diafenthiuron 50% WP @ 600g ha^{-1}, and an untreated check.

RESULTS AND DISCUSSION

The results revealed that all the treatments were significantly superior over untreated check in reducing the incidence of *A. gossypii*; imidacloprid 6%+ lambda cyhalothrin 4% SL@ 500 ml ha^{-1}, 450 ml ha^{-1} and 400 ml ha^{-1} were found to be significantly superior over all other treatments and on par with each other by recording 89.02, 86.81, 85.71 in first season and 93.03, 91.18 and 88.47% reduction of *A. gossypii* incidence over untreated check in second season, respectively. Diafenthiuron 50% WP @ 600 g ha^{-1} (45.50 & 58.90 %) was found to be the least effective against *A. gossypii*. Imidacloprid 6%+ lambda cyhalothrin 4% SL @ 500 ml ha^{-1}, 450 ml ha^{-1} and 400 ml ha^{-1} performed well in reducing the incidence of *T. tabaci* on cotton, which were on par with each other and superior over all other treatments. Buprofezin 25% SC @ 1000 ml ha^{-1} and imidacloprid70% WG @ 35 g ha^{-1} were effective next to the imidacloprid 6%+ lambda cyhalothrin 4% SL @ 500 ml ha^{-1}, 450 ml ha^{-1} and 400 ml ha^{-1} (Table 1). The mean data of incidence of *A. biguttula biguttula* revealed that imidacloprid 6%+ lambda cyhalothrin 4% SL @ 500 ml ha^{-1}, 450 ml ha^{-1} and 400 ml ha^{-1} was the most effective. It was followed by buprofezin 25% SC @ 1000 ml ha^{-1} (78.96 %), imidacloprid 6%+ lambda cyhalothrin 4% SL @ 350 ml ha^{-1} (73.21%), imidacloprid 70% WG @ 35 g ha^{-1} (64.99 %), lambda cyhalothrin 5% EC @ 500 ml ha^{-1} (63.17 %) and diafenthiuron 50% WP @ 600 g ha^{-1} (30.11%) in first season. The similar trend was noticed in second season as well. Against *B. tabaci* all the treatments were significantly superior over untreated check; imidacloprid 6%+ lambda cyhalothrin 4% SL @ 500 ml ha^{-1} gave 90.17% reduction in first season followed by imidacloprid 6%+ lambda cyhalothrin 4% SL @ 450 ml ha^{-1} (89.31 %), imidacloprid 6%+ lambda cyhalothrin 4% SL @ 400 ml ha^{-1} (89.78 %), imidacloprid 6%+ lambda cyhalothrin 4% SL @ 350 ml ha^{-1} (81.83%), imidacloprid 70% WG @ 35 g ha^{-1} (70.30 %). In second season, the similar trend was noticed (Table 1).

All the tested insecticides showed non-significant differences and were statistically on par with each other and found to be safer towards natural enemies viz., coccinellids and chrysopids (Table 2). The present findings are in line with Ameta and Sharma (2005) indicated that imidacloprid 70 WG at 30 and 35 g ha^{-1} were effective against sucking pests of cotton. Saleem et al. (2001) reported that Confidor 200 SL effectively controlled sucking pests up to seven days after the spray in cotton. Asif et al. (2016) reported that imidacloprid and lambda cyhalothrin were effective against cotton sucking pests. Pal et al. (2020) reported that combination product of imidacloprid 6% + lambda cyhalothrin 4%SL @ 500 ml/ha and 450 ml/ha proved the most effective in Bt cotton. Significantly highest seed cotton yield was recorded in the plots treated with imidacloprid 6%+ lambda cyhalothrin 4% SL @ 500 ml ha^{-1} and was on par with its lower doses at 450ml/ ha and 400 ml/ ha^{-1} (Table 2). The effectiveness of imidacloprid on the increase in yield was already reported by Gupta et al. (2005) and Ameta and Sharma (2005) on cotton. Similarly, effectiveness of lambda cyhalothrin on the increase in yield was reported by Asif et al. (2016).

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AUTHOR CONTRIBUTION STATEMENT

SV Hugar conducted the experiments and SS Udikeri prepared the manuscript, all authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

Table 1. Efficacy of Imidacloprid 6%+ Lambda cyhalothrin 4% SL against sucking pest complex in cotton

S. No.	Treatments	Dose (g a.i. / ha)	Mean incidence of sucking pests/ three leaves							
			Kharif-2018-19 (First season)				Kharif-2019-20 (Second season)			
			Aphids	Thrips	Leafhoppers	Whitefly	Aphids	Thrips	Leafhoppers	Whitefly
T_1	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	350 ml	16.26 (4.09)	20.14 (4.54)	6.06 (2.56)	5.06 (2.36)	14.53 (3.88)	21.39 (4.68)	5.86 (2.52)	7.14 (2.76)
T_2	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	400 ml	11.94 (3.53)	15.66 (4.02)	3.82 (2.08)	3.80 (2.07)	10.13 (3.26)	15.79 (4.04)	3.22 (1.93)	5.06 (2.36)
T_3	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	450 ml	10.59 (3.33)	14.62 (3.89)	3.44 (1.99)	3.54 (2.01)	8.79 (3.05)	14.35 (3.85)	2.73 (1.80)	3.80 (2.07)
T_4	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	500 ml	9.34 (3.14)	13.46 (3.74)	3.36 (1.96)	3.42 (1.98)	7.66 (2.86)	13.00 (3.67)	2.04 (1.59)	3.54 (2.01)
T_5	Lambda cyhalothrin 5% EC	500 ml	19.97 (4.52)	24.36 (4.99)	8.82 (3.05)	6.69 (2.68)	16.90 (4.17)	23.49 (4.90)	6.79 (2.70)	6.58 (2.66)
T_6	Imidacloprid 70% WG	35 g	17.67 (4.01)	21.79 (4.50)	7.30 (2.54)	7.44 (2.82)	11.77 (3.50)	18.95 (4.41)	4.67 (2.27)	6.69 (2.68)
T_7	Buprofezin 25% SC	1000 ml	15.61 (4.26)	19.72 (4.72)	5.96 (2.79)	7.14 (2.76)	13.26 (3.71)	17.78 (4.28)	4.67 (2.27)	7.44 (2.82)
T_8	Diafenthiuron 50% WP	600 g	22.87 (4.83)	27.23 (5.27)	11.49 (3.46)	8.90 (3.07)	19.75 (4.50)	26.98 (5.24)	8.56 (3.01)	8.90 (3.07)
T_9	Control	--	36.80 (6.11)	43.07 (6.60)	13.77 (3.78)	14.38 (3.86)	33.90 (5.87)	44.88 (6.74)	12.69 (3.63)	14.38 (3.86)
	SEm ±		0.20	0.16	0.08	009	0.18	0.19	0.07	0.10
	CD (p=0.05)		0.62	0.48	0.24	0.27	0.54	0.56	0.21	0.30
	CV (%)		8.63	5.92	5.20	5.96	8.17	7.03	5.14	6.57

Mean = Mean of observations 14 days after spray; Figures in parentheses$\sqrt{x + 0.5}$ transformed values.

Table 2. Efficacy of Imidacloprid 6%+ Lambda cyhalothrin 4% SL on natural enemies and yield in cotton

S. No.	Treatments	Dose (g a.i. / ha)	Season I		Season II		Seed cotton yield (Q/ ha)	
			Mean Coccinellids/ 5 plants	Mean Chrysopids/ 5 plants	Mean Coccinellids/ 5 plants	Mean Chrysopids/ 5 plants	Season I	Season II
T_1	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	350 ml	3.56 (2.01)	4.04 (2.13)	3.56 (2.01)	3.88 (2.09)	13.58	13.02
T_2	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	400 ml	3.30 (1.95)	3.69 (2.05)	4.04 (2.13)	3.52 (2.00)	15.64	15.06
T_3	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	450 ml	3.13 (1.90)	4.36 (2.20)	3.69 (2.05)	3.59 (2.02)	15.95	15.32
T_4	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	500 ml	3.39 (1.97)	4.07 (2.14)	4.36 (2.20)	4.13 (2.15)	16.33	15.62
T_5	Imidacloprid 6%+ Lambda cyhalothrin 4% SL	500 ml	3.38 (1.97)	4.14 (2.15)	4.07 (2.14)	3.16 (1.91)	13.71	13.00
T_6	Imidacloprid 70% WG	35 g	3.72 (2.05)	4.36 (2.20)	4.14 (2.15)	3.55 (2.01)	13.41	13.12
T_7	Buprofezin 25% SC	1000 ml	3.95 (2.11)	3.70 (2.05)	4.36 (2.20)	3.82 (2.08)	14.24	14.07
T_8	Diafenthiuron 50% WP	600 g	3.93 (2.11)	3.76 (2.06)	3.70 (2.05)	3.68 (2.05)	13.26	13.02
T_9	Control	--	4.42 (2.22)	4.54 (2.24)	4.57 (2.25)	6.04 (2.56)	11.02	10.60
SEm ±			--	--	--	---	0.50	0.65
CD (p=0.05)			NS	NS	NS	NS	1.52	1.96
CV (%)			--	--	--	--	6.59	7.89

Mean = Mean of observations 14 days after spray; Figures in parentheses $\sqrt{x + 0.5}$ transformed values.

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INSECT VECTORS ASSOCIATED WITH VIRAL DISEASES OF KING CHILLI (*CAPSICUM CHINENSE* JACQ.) IN NORTH EAST INDIA

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ABSTRACT

A transmission study was conducted to identify and confirm the insect vectors of viral diseases of king chilli *Capsicum chinense* Jacquin, at the Virology Laboratory, Assam Agricultural University, Jorhat. The study reveals that Cucumber Mosaic Virus (CMV), Potato Virus Y (PVY) and Chilli Leaf Curl Virus (ChLCV) were the viral diseases transmitted by *Aphis gossypii* (Glover), *Myzus persicae* (Sulzer) and *Bemisia tabaci* (Gennadius), respectively. The detection of CMV and PVY were done through DAS-ELISA assay and ChLCV was done through PCR technique. The results showed that *A. gossypii* and *M. persicae* were successfully transmitting the CMV and PVY (40% each). DAS-ELISA revealed that 57.14% of the plant and 66.67% of *A. gossypii* samples were found to be positive to CMV. While, 42.17 and 60.00 % of PVY detection was observed from plant and *M. persicae* samples, respectively. *B. tabaci* also successfully transmitted ChLCV with 80% of the tested plant and the PCR results revealed that the primer pair ChLCV F1 and ChLCV R1 successfully yields 550bp at annealing temperature of 48°C from the infected plant as well as from *B. tabaci* samples and fail to detect from healthy plants, aphids, and leafhoppers samples.

Key words: *Capsicum chinense*, viral diseases, CMV, PVY, ChLCV, *Aphis gossypii*, *Myzus persicae*, *Bemisia tabaci*, vectors, transmission, DAS-ELISA, PCR, detection

North East India is one of the prominent hot spots for biodiversity, and king chilli *Capsicum chinense* Jacquin, one of the hottest chilli is extensively grown here with high benefit-cost ratio (Thangjam et al., 2021). However, one of the challenging problems in chilli cultivation is viral disease complex and chilli leaf curl disease is one of these (Shih et al., 2006; Meena et al., 2008). Kenganal et al. (2006) also reported that >20 viruses were found to infect chilli, of which mosaic and leaf curl diseases were the most devastating viral diseases. King chilli is highly susceptible to viral disease complex showing symptoms like stunted growth, leaf mosaic, leaf mottling, leaf curl, deformed leaf and deformed fruits which significantly hamper its production and productivity. Talukdar et al. (2015) identified 4 viral diseases based on symptomatology, transmission, host range and serological assays. These diseases are caused by Cucumber Mosaic Virus (CMV), Potato Virus Y (PVY), Tomato Spotted Wilt Virus and Chilli Leaf Curl Virus (ChLCV) from Assam. However, limited information was available on insect vectors aspects and therefore, the present study to identify the vectors and confirmation of these viruses through serological and molecular methods.

MATERIALS AND METHODS

To identify the vectors of viral diseases and confirmation of these viruses, transmission studies were carried out in the Virology Laboratory at the Department of Plant Pathology, Assam Agricultural University, Jorhat, during 2016-17. About 20 plants were grown in the plastic pot to study the transmission studies. The insect vectors viz., aphids (*Aphis gossypii* and *Myzus persicae*) and whitefly (*Bemisia tabaci*) were collected from the field using brush and aspirator, and then separately reared and maintained on healthy brinjal and king chilli plants in insect-proof cages for five generations and maintained non-viruliferous before transmission studies. The transmission study of CMV and PVY were conducted following standard procedure given in Garzo et al. (2004) and *A. gossypii* and *M. persicae* were used for transmission. For ChLCV, *B. tabaci* was used as vector following Butter and Rataul (1977) with little modification. After inoculation, the viruliferous vectors were killed by spraying with imidacloprid @ 0.5ml/ l of water. The plants were maintained for 30 days inside insect-proof cages and were inspected daily for symptom development. For

detection of viruses through serological and PCR methods, the samples were collected from experimental fields as well as from Laboratory. Double antibody sandwich enzyme linked immonosorbent assay (DAS-ELISA) of both the plant samples as well as aphids were performed following methods described by Clark and Adams (1977) to detect CMV and PVY. The antibodies were obtained from Bioreba, AG, CH4153, Reinach BL1, Switzerland. After the addition of substrate, detection was performed after 60 minutes at 405nm in an ELISA reader (Bio-Rad Instruments Inc). The samples were considered infected with virus if ELISA reading was three times above the average reading of the control and the two blanks.

For detecting ChLCV from both plant samples and insect vectors, PCR technique was followed. CTAB (Cetyl Trimethyl Ammonium Bromide) method of Kollar et al. (1990) was performed to isolate genomic DNA from plants samples and Phenol-Chloroform extraction protocol of Barr et al. (2009) was followed for insect vectors with little modification. A pair of primers (ChLCV1) specific to the coat protein region of PepLCV genome sequence (GenBank accession no. EF 190217) was used for PCR to amplify the ChLCV coat protein gene as described by Sinha et al. (2011). The forward primer (5' AGAATTATGTCCAAGCGACCA 3') and the reverse primer (5'AAGCGTTGGGGATACACAAA 3') having an amplicon size of 550 base pair was used for amplification of king chilli leaf curl virus. PCR was performed in 25 µl volume using primer pair ChLCV1. The reaction mixture consisted of 2.5 µl 10X PCR buffer (with 17.5 mM MgCl2), 2.0 µl of dNTPs, 2.0 µl of forward and reverses primers, 0.9 µl Taq DNA polymerase (3U/ µl), 2.0 µl template (50ng/ µl), 13.6 µl of nuclease free water. For primer pair, ChLCVF1 – ChLCVR1 DNA amplification parameters were 40 cycles of initial denaturation for 3 min at 94°C, denaturation for 1 min at 94°C, annealing for 1 min at 48°C, extension for 1 min at 72°C and final extension at 72°C for 10 min. Amplification products were hold at 4°C for infinity prior to gel electrophoresis. The annealing temperature for primer ChLCV was optimized by using gradient PCR. The gradient PCR experiment was performed at 46- 48°C viz., 46, 47 and 48°C with a reaction mixture of 25 µl. The PCR products were electrophoresis at 50 mAmp in BIO-RAD electrophoretic apparatus with 1.2% agarose gel stained with ethidium bromide and visualized under UV trans-illuminator and captured the gel images using the geldoc (Alpha Innotech, USA) for documentation.

RESULTS AND DISCUSSION

The transmission studies of viral diseases in king chilli reveals that the aphids; *A. gossypii* and *M. persicae* could successfully transmit CMV and PVY respectively, showing symptoms of stunted growth, yellow mosaic, mottling, deformed leaves and fruits. *A. gossypii* and *M. persicae* were transmitting the viruses up to 40 % of the plants. The initial symptoms expression of CMV takes about 14 to 15 days after inoculation, whereas in case of PVY, it takes about 9 to 10 days. The finding was in conformity with Talukdar et al. (2015), who observed successful transmission of CMV and PVY through *A. gossypii* and *M. persicae* in *Bhut Jolokia.* However, Baruah et al. (2016) observed that the aphids could not transmit PVY in *Bhut Jolokia* plant, whereas CMV was successfully transmitted through mechanical method after 14 days of incubation period in Assam. A similar result was also reported by Biswas et al. (2013). The whitefly (*B. tabaci*) successfully transmitted the ChLCV to king chilli up to the extent of 80 % during the transmission study. The leaves were deformed, elongated, and curled upward and downward. The fruits that bear were also small and deformed. The symptoms were started to be expressed to the inoculated plant after 18 to 23 days (Table 1). Similar results were also

Table 1. Transmission efficiency of insect vectors on CMV, PVY and ChLCV, respectively on king chilli

Insect vector	Virus	No. of vector released per plant	Acquisition access period	Inoculation access period	No. of plants		% Infection	Days to symptoms (days)	Average symptom appearance (days)
					Inoculated	Symptomatic			
Aphis gossypii	CMV	10	5 min	2 hr	5	2	40.00	14-15	14.50
Myzus persicae	PVY	10	5 min	2 hr	5	2	40.00	9-10	9.50
Bemisia tabaci	ChLCV	10	24 hrs	5 hr	5	4	80.00	18-23	20.50

**CMV= Cucumber mosaic virus, PVY= Potato virus Y and ChLCV= Chilli leaf curl virus

reported by Senanayake et al. (2006), who observed 100% successful transmission on the release of 8 or more whiteflies in chilli, and Baruah et al. (2016) also found 50% successful transmission with whitefly in Bhut Jolokia. Pandey et al. (2010) also reported that chilli leaf curl is transmitted by whitefly (*B. tabaci*) and inoculated chilli plants showed typical leaf curl symptoms after 2-6 weeks.

The results of DAS-ELISA assay revealed that 57.14 % of the plant and 66.67% of *A. gossypii* samples were found to be positive to CMV, while in the case of PVY, 42.17 and 60.00% of detection was recorded from plant and *M. persicae* samples, respectively (Table 2). Similar results were reported by Talukdar et al. (2017) who reported that 87.5% of Potyvirus and 75.0% of cucumovirus was found to be positive in Bhut Jolokia through DAS-ELISA and Baruah et al. (2016) also found 55.0% infection of CMV and 36.0% of PVY in Bhut Jolokia samples through DAS-ELISA assay in Assam. The PCR results revealed that the primer pair ChLCVF1 and ChLCVR1 successfully yielded a 550bp PCR product from 35.56% of the plant samples tested and 33.33% from vector *B. tabaci* out of three samples tested but failed to detect from healthy plants, aphids, and leafhoppers samples using the same primer (Table 2; Fig. 1). The results were in conformity with Adluri et al. (2017) that detect the leaf curl virus from the infected plants of *Bhut Jolokia* by using the primer pair ChLCV1, and a similar result was also reported by Sinha et al. (2011) from the pepper. The king chilli is highly susceptible to viral disease complex which is one of the significant constraints that hamper its production and productivity which are transmitted by sucking pests. From the present study, it is found that the Cucumber

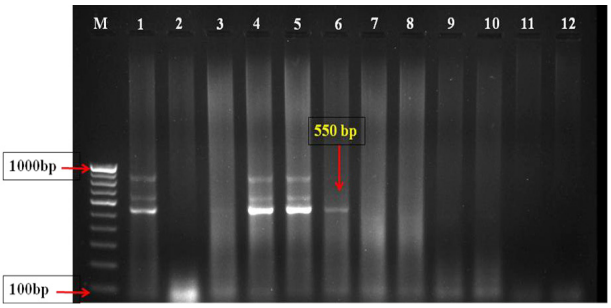


Fig. 1. ChLCV amplified product on agarose gel using ChLCV specific primer. M: 100bp Marker, 1: +ve Sample, 2: -ve Sample, 3: Healthy plant, 4-5: Infected Plant, 6: Whitefly, 7-8: Aphids, 9-12: Leaf hoppers

Mosaic Virus (CMV) and Potato Virus Y (PVY) are transmitted by aphids (*A. gossypii* and *M. persicae*) and it is confirmed by conducting DAS-ELISA assay of both the vectors and plants samples. Similarly, the whitefly (*B. tabaci*) is a vector of ChLCV in king chilli and it is also confirmed through PCR techniques. Hence, these techniques are very accurate and helpful in detecting and identifying the vectors of viral diseases and managing the viral diseases to a considerable limit.

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Table 2. Detection of viruses from king chilli and its vectors through DAS –ELISA and PCR technique

Detection	Samples	Number of samples tested	Number of positive samples	Per cent detection	Range of ELISA value (OD_{405})	Mean positive OD_{405}
CMV	King chilli	126	72	57.14	0.102 - 0.563	0.431
	A. gossypii	6	4	66.67		
PVY	King chilli	83	35	42.17	0.113 - 0.368	0.294
	M. persicae	5	3	60.00		
ChLCV	King chilli	180	64	35.56	-	-
	Bemisia tabaci	3	1	33.33		
	Aphids	3	0	0.00		
	Leafhoppers	3	0	0.00		

**Cut off value for ELISA positive sample for CMV = 0.420 and PVY =0.194

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REPORT OF FALL ARMY WORM *SPODOPTERA FRUGIPERDA* FROM BUNDELKHAND REGION OF UTTAR PRADESH

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ABSTRACT

The fall army worm (FAW) *Spodoptera frugiperda* (J E Smith) is spreading throughout maize growing tracts of India. In the Bundelkhand region of Uttar Pradesh this pest was noticed in Banda district during 2020. The maize crop was badly affected in the region and even harvesting could not be possible because of the heavy toll caused by the FAW larvae in production of cobs. Finger millet and groundnut were other crops which were severely affected.

Key words: *Spodoptera frugiperda,* Banda, Bundelkhand region, damage, incidence, finger millet, groundnut, maize, Uttar Pradesh

The Bundelkhand region has semiarid landscape and the cropping pattern is monsoon based, with sorghum, maize, bajra, minor millets, pigeonpea, mung bean, urd bean, groundnut, linseed, sesame and vegetables grown during kharif season. These are heavily infested by insect pests with economic losses. The kharif crops viz., rice, maize, mungbean, urd bean, pigeonpea, sesame, groundnut, finger millet are grown in the university farm. Routine field inspection of these revealed occurrence of many caterpillars. Considering the damage severity, a study was done to ascertain the status of fall army worm *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae). This pest has gained attention internationally since its outbreak in Africa in 2016 and Asia in mid-2018. It is found in the western hemisphere's tropical zone, from the United States to Argentina. It was discovered in Africa in 2016, inflicting severe damage to maize (Goergen et al., 2016). It has a wide host range including maize, sorghum, millets, rice, sugarcane, soybean, vegetables, and cotton (Prowell et al., 2004; Bueno et al., 2010; Padhee and Prasanna, 2019). The occurrence of *S. frugiperda* and its associated natural enemies is reported from India (Shylesha et al., 2018; Sharanabasappa et al., 2018). This study explores its occurrence from the Bundelkhand region of Uttar Pradesh.

MATERIALS AND METHODS

The study was conducted in the field and laboratory of Banda University of Agriculture and Technology, Banda. The various crops grown were inspected to observe the incidence of insect pests, from July to October, 2020. Under a routine inspection, it was noticed that a caterpillar infested entire maize fields and adjoining areas covering finger millet and groundnut. Scheduled visits were made at 10 days interval, and the pest was identified, with observations on symptoms of damage. Its morphological features of different stages were studied in the laboratory. Key characters were observed as described earlier (Oliver and Chapin, 1981; Prasanna et al., 2018; Sharanabasappa et al., 2018; Ganiger et al., 2018). Larvae collected from fields were reared in laboratory to confirm its biology..

RESULTS AND DISCUSSION

The damage observed in the fields revealed that leaves had longitudinal scars as well as irregular holes along with torned leaves. The lumps of excrement of larva were observed on the leaves as well as whorls of infested plants. The infested crop was at vegetative stage and more damage was noticed on the crops sown 15-20 days during July. Deshmukh et al. (2021) observed initially, first instar larva scraps the chlorophyll from the leaves and make a longitudinal scar. However, second and third instar larvae make holes in tightly twisted whorls of the maize. Shylesha et al. (2018) also reported that in the later stages, larval feeding creates huge feeding patches on the open leaves. It was observed that larvae were usually hidden during the daytime and come out at night. It was observed that during kharif season, a number of crops viz., rice, maize, finger millet, groundnut, sesame, pigeonpea, mungbean, urdbean, etc. were grown in the farm of the University. Of these, severe infestation was observed

in maize, finger millet and groundnut. This pest is a polyphagous pest of voracious nature, with a large host range of >100 plant species in 27 families (Goergen et al., 2016). It prefers Gramineae plants, which include maize, millet, sorghum, sugarcane, rice and wheat (Shylesha et al., 2018).

The larval and adult stages collected and reared in the laboratory revealed that the eggs were laid in groups with a layer of gray over the egg mass. The hatched larvae were in groups, green, with black heads, while later instars were brown with white lines on the either side of the body. The head of the mature larva was reddish brown and there were dark spots on the body. In addition, a white inverted 'Y' mark observed on the front of the face of the mature larva as well as four visible black dots grouped in a square on the dorsum of the eighth abdominal segment. Fully grown larva underwent pupation with the remnants of dried leaves and other materials. Pupa was reddish brown with two spines on the cremaster. Adult moths emerged after 8 to 10 days of pupation. Moths were 35 to 38 mm in size, with fore wing of the male brown and the triangular white spots are at the apex and center of the wing; in female moth these marks were not evenly observed; but with variegated silver-white mark with narrow dark borders in both male and female. These findings corroborate with those of Oliver and Chapin, 1981; Prasanna et al., 2018; Sharanabasappa et al., 2018; Ganiger et al., 2018; Shylesha et al., 2018; Gavas and Balan, 2020. Moths were most active in the evening and during the night hours. Infestation in maize and other crops at Banda has been thus confirmed. IITA (2018) has raised greater concern about the invasion of *S. frugiperda* into India; it has also been reported in maize growing areas from Aligarh region of Uttar Pradesh (Shekhawat, 2019; Naganna et al., 2020).

AUTHOR CONTRIBUTION STATEMENT

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CONFLICT OF INTEREST

No conflict of interest.

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HOST RANGE OF COCONUT RUGOSE SPIRALLING WHITEFLY *ALEURODICUS RUGIOPERCULATUS* MARTIN

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ABSTRACT

Coconut (*Cocos nucifera* L.) is attacked by a wide variety of insect pests of which rugose spiralling whitefly (RSW), *Aleurodicus rugioperculatus* Martin has been causing widespread damage in growing major coconut growing districts of Tamil Nadu since 2016 and has been recorded as an invasive pest in coconut. The pest is expanding its host range in economic field and horticultural crop plants besides weed fauna, posing a real threat to farming community. Extensive survey undertaken in Tamil Nadu during 2018 to 2020 to study the expanding host range of RSW which revealed the presence of the pest in about 67 host plants including coconut. Management of the pest through chemical means is highly difficult and the parasitoid, *Encarsia guadeloupae* has been observed to effectively suppress the pest. Host plants that harbor more *Encarsia* parasitoids (which are referred as banker plants) would help in biosuppression of the pest. Upon studying the host range, it was found that, the RSW was able to complete its life cycle and successfully emerge as adults in only six hosts viz., coconut, teak, banana, guava, maize and Spanish cherry, while five other hosts viz., *Annona muricata, A. squamosa, A. reticulata, Theobroma cacao* and *Ficus religiosa* supported RSW up to its nymphal stage and harboured *Encarsia guadeloupae* parasitoids in larger numbers. These hosts can be used as banker crops in and around coconut plantations so that the emerging parasitoids will aid in biosuppression of the target pest.

Key words: *Aleurodicus rugioperculatus*, host range, *Encarsia guadeloupae*, banker plants, *Annona muricata, A. squamosa, A. reticulata, Theobroma cacao, Ficus religiosa,* natural suppression

Coconut (*Cocos nucifera* L.) is attacked by a wide variety of insect pests of which the invasive pest, rugose spiralling whitefly (RSW) *Aleurodicus rugioperculatus* Martin has been causing widespread damage in growing major coconut districts of Tamil Nadu since 2016. The species was first reported in coconut (*Cocos nucifera* L.) during 2004 in Belize, Central America (Martin, 2004), South Florida, United States in 2009 (Stocks and Hodges, 2012) and in Pollachi tract, Tamil Nadu, Coimbatore district, Tamil Nadu during August, 2016 (Shanas et al., 2016; Srinivasan et al., 2016; Sundararaj and Selvaraj, 2017; Selvaraj et al. 2017). Subsequent to its identification and confirmation, the rugose spiralling whitefly (RSW) tended to show its presence in other economic plants and weeds, thus posing a grave danger to the field and horticultural crop plants. The RSW has been known to attack about 118 hosts including cultivated crops and weed flora (Stocks and Hodges, 2012). The vast host range reported by Stocks and Hodges (2012) showed this pest is more attracted to ornamental plants, thus evincing not much interest in the minds of field and horticultural crop researchers. Thus, literature on the RSW was sparse and in particular, the host range of the species was poorly studied in South East Asia.

With regard to host range, RSW has been identified on 118 plant species including edibles, ornamentals, palms, weeds as well as native and invasive plant species (Francis et al., 2016). Surveys by Shanas et al. (2016) revealed the infestation of RSW in about 17 hosts while Srinivasan et al. (2016) reported a host range of 15 plant species. Preliminary studies conducted by Srinivasan et al. (2016) highlighted a nymphal parasitoid, *Encarsia guadeloupae* Viggiani (Hymenoptera: Aphelinidae), that was providing excellent biosuppression of RSW which have also been reported by Ramani et al. (2002) and Evans (2007) but against *Aleurodicus disperses*. The parasitoid has already been reported on *A. rugioperculatus* from Florida (Kumar et al., 2013) and appears to be a potential biocontrol agent against RSW as 50 to 60% natural parasitisation of the pupae was observed (Shanas et al., 2016). Hence attempts to augment these parasitoids in infested coconut gardens

was made by collecting and redistributing the coconut leaves with parasitized nymphs as an inoculative release in the infested coconut gardens. During the surveys in Coimbatore and Tiruppur districts, the occurrence of *Encarsia* was noticed in hosts other than coconut and banana. These hosts considered as "banker plants" (that invite more of parasitoids and help in natural biosuppression of pest species in crop ecosystems) (Gurr et al., 2017) assume greater importance in the present era of non-chemical pest control. Thus, the present study is intended to explore the host range of RSW, and in the process, identifying hosts that could serve as banker plants for aiding natural suppression of the pest.

MATERIALS AND METHODS

Survey was taken up in the host plants found in the vicinity of coconut plantations in Tamil Nadu Agricultural University campus (11.022 N,76.927 E) and in coconut gardens in Tamil Nadu during 2018 to 2020. For a particular host plant, a minimum of 25 plants were examined to ascertain the presence of RSW. The host plants were photo-documented using a portable handheld camera. The plants that were considered as host plants include those that were found laden with all the life stages of *A. rugioperculatus*. The host plants were differentiated into three groups *viz.,* hosts that housed the egg stages and exhibited lack of further development to nymphal stages; hosts that supported up to nymphal stages only where adult emergence failed and hosts that supported the full development of RSW life stages and exhibiting eggs, nymphal and adult colonies of RSW. Consequent to the recording of the host plants and the stage of RSW in the respective host plant, additional observations on the parasitism by *Encarsia guadeloupae* Viggiani was recorded. This was done by collecting 10-15 leaf samples of the host plant along with the nymphal stage and observing under the microscope. The RSW nymphs showing circular emergence holes are the ones from which the *E. guadeloupae* adults have emerged. Among such colonies were found blackened nymphal stages which represent RSW nymphs already parasitized by *E. guadeloupae*. The host plants that house RSW nymphs along with this type of emergence holes/ blackened nymphs and those housing adult parasitoids were also recorded.

RESULTS AND DISCUSSION

The survey for rugose spiralling whitefly (RSW) in and around coconut gardens of TNAU campus revealed that RSW life stages were observed in 67 host plants including coconut (Table 1). Regarding the host range of the RSW, Francis et al. (2016) reported about 118 host plants belonging to 43 families in the United States. Stocks and Hodges (2012) reported about 95 host plants in Florida, USA. Studies by Shanas et al. (2016) highlighted 12 plant species as host of *A. rugioperculatus* in Kerala. In Tamil Nadu a total of 15 hosts belonging to 13 families have been reported by Srinivasan et al. (2016). About 9 hosts were recorded by Selvaraj et al. (2017). Out of the hosts recorded during the present studies, 7 belonged to each Fabaceae, 6 in Malvaceae, 5 in Euphorbiaceae, 4 each in Annonaceae and Sapotaceae and 3 each in Araceae, Meliaceae, Poaceae and Rutaceae. Apart from these 26 other families were recorded. A classification on the basis of economic importance of the host plants revealed that the RSW was able to inflict damage in a range of economically important crops. Of the 67 host plants recorded, 17 fell under the ornamental plants category, followed by 14 under fruit crops, 9 under medicinal plants, 5 under vegetables, 4 under fibre crops, 2 each under biofuels, flower crops, green leaf manures, millets, oilseeds, plantation crops and timber crops, besides 1 host each under pulses, avenue trees, fodder crop and tuber crops.

The invasive RSW was first recorded in Tamil Nadu during 2016 (Srinivasan et al., 2016). Within a short span of time i.e. 2 years since its entry, the pest had its host range expanded to about 67 hosts which is a sign of the ability of RSW to survive on different host plants. The expanding host range of RSW should be eyed with greater caution, as their infestation in export-oriented crops may bring various problems in the international trade arena. The ability of RSW to inflict damage in a range of economically important crops viz*.,* ornamental plants, fruit crops, medicinal plants, vegetables, fibre crops, biofuels, flower crops, green leaf manures, millets, oilseeds, plantation crops, timber crops, pulses, avenue trees, fodder crop and tuber crops indicates the pest's invasive ability within a short span of time. Among the 67 host plants, only six hosts supported the full growth and development of RSW *viz*., *Cocos nucifera, Tectona grandis, Musa paradisiaca, Psidium guajava, Zea mays* and *Mimusops elengi,* while five other hosts *viz., Annona muricata, A. squamosa, A. reticulata, Theobroma cacao* and *Ficus religiosa* supported RSW up to its nymphal stage and the remaining 56 hosts were preferred only for oviposition by RSW.

Table 1. Host plants and parasitism of *A. rugioperculatus*

Common name	Scientific name	Family	Economic importance	Life stages	Parasitism by *Encarsia guadeloupae*
Vilvam/ bael	*Aegle marmelos* L.	Rutaceae	Medicinal	E	-
Neem	*Azadiracta indica* L.	Meliaceae	Medicinal	E	-
Camel foot	*Bauhinia purpurea* L.	Fabaceae	Ornamental	E	-
Orchid tree	*Bauhinia sp.* L.	Fabaceae	Ornamental	E	-
Alexandrian laurel	*Calophyllum inophyllum* L.	Clusiaceae	Medicinal	E	-
Jack bean	*Canavalia ensiformis* L.	Fabaceae	Vegetable	E	-
Karanda	*Carissa carandas* L.	Apocynaceae	Fruits	E	-
Star apple	*Chrysophyllum cainito* L.	Sapotaceae	Fruits	E	-
Citrus	*Citrus limon* L.	Rutaceae	Fruit	E	-
Ivy gourd	*Coccinea grandis* L.	Cucurbitaceae	Vegetable	E	-
Sunnhemp	*Crotolaria juncea* L.	Fabaceae	Green manure	E	-
Purple yam	*Dioscorea alata* L.	Dioscoreaceae	Vegetable	E	-
Wood apple	*Feronia elephantum* Corr.	Rutaceae	Fruit	E	-
Banyan	*Ficus benghalensis* L.	Moraceae	Ornamental	E	-
Fernleaf tree	*Filicium decipiens* Wight & Arn.	Sapindaceae	Ornamental	E	-
Gliricidia	*Gliricidia sepium Jacq.*	Fabaceae	Green leaf manure	E	-
Wild cotton	*Gossypium armourianum* Kearn.	Malvaceae	Fibre	E	-
Wild cotton	*Gossypium gossypioides Standley*	Malvaceae	Fibre	E	-
Cotton	*Gossypium hirsutum* L.	Malvaceae	Fibre	E	-
Wild cotton	*Gossypium robinsonii* F. Muell.	Malvaceae	Fibre	E	-
Sunflower	*Helianthus annuus* L.	Asteraceae	Oilseeds	E	-
Hibiscus	*Hibiscus rosa- sinensis* L.	Malvaceae	Ornamental	E	-
Indian elm	*Holoptelea integrifolia* Roxb.	Ulmaceae	Ornamental	E	-
Morning glory	*Ipomoea purpurea* L.	Convolvulaceae	Ornamental	E	-
Jatropha	*Jatropha curcas* L.	Euphorbiaceae	Biofuel	E	-
Spicy Jatropha	*Jatropha integerimma* Jacq.	Euphorbiaceae	Medicinal	E	-
Malabar nut	*Justicia adhatoda* L.	Acanthaceae	Medicinal	E	-
Lab lab	*Lablab purpureus* L.	Fabaceae	Pulses	E	-
Mahua	*Madhuca longifolia* L.	Sapotaceae	Medicinal	E	-
Mango	*Mangifera indica* L.	Anacardiaceae	Fruits	E	-
Tapioca	*Manihot esculenta* L.	Euphorbiaceae	Tuber	E	-
Sapota	*Manilkara zapota* L.	Sapotaceae	Fruits	E	-
Green Malabar Neem	*Melia dubia* Cav.	Meliaceae	Medicinal	E	-
Indian Beech Tree	*Milletia pinnata* L.	Fabaceae	Biofuel	E	-
Noni	*Morinda citrifolia* L.	Rubiaceae	Medicinal	E	-
Moringa	*Moringa oleifera* L.	Moringaceae	Vegetable	E	-
Nerium	*Nerium oleander* L.	Apocynaceae	Flower	E	-
Proso millet	*Panicum miliaceum* L.	Poaceae	Millets	E	-
Avocado	*Persea americana* Mill.	Lauraceae	Fruits	E	-
Lacy tree	*Philodendron bipinnatifidum* Schott.	Araceae	Ornamental	E	-
Philodendron Lemon lime	*Philodendron hederaceum var aureum*	Araceae	Ornamental	E	-

(contd.)

Indian gooseberry	*Phyllanthus emblica* L.	Phyllanthaceae	Fruits	E	-
False Asoka tree	*Polyalthia longifolia* Sonn.	Annonaceae	Avenue tree	E	-
Money plant	*Pothos sp.* L.	Araceae	Ornamental	E	-
Castor	*Ricinus communis* L.	Euphorbiaceae	Oilseed	E	-
Rose	*Rosa* sp. L.	Rosaceae	Flower	E	-
Indian sandal wood	*Santalum album* L.	Santalaceae	Timber	E	-
Soapnut	*Sapindus emarginatus* Vahl.	Sapindaceae	Medicinal	E	-
Asoka tree	*Saraca asoca* Roxb.	Caesalpinioideae	Ornamental	E	-
Chekurmanis	*Sauropus androgynous* (L.) Merr.	Euphorbiaceae	Vegetable	E	-
Paradise tree	*Simarouba glauca* DC.	Simaroubaceae	Ornamental	E	-
Mahogany	*Swietenia macrophylla* King.	Meliaceae	Ornamental	E	-
Jamun	*Syzigium cumini* (L.) Skeels	Myrtaceae	Fruits	E	-
Yellow bells	*Tecoma stans* (L.) Juss.	Bignoniaceae	Ornamental	E	-
Tree marigold	*Tithonia diversifolia* (Hemsl.) A. Gray	Asteraceae	Ornamental	E	-
Cumbu Napier hybrid	*Pennisetum glaucum* L. x *Pennisetum purpureum* Schum.	Poaceae	Fodder	E	-
Sour sop	*Annona muricata* L.	Annonaceae	Fruits	E, N	+++
Custard apple	*Annona reticulata* L.	Annonaceae	Fruits	E, N	++
Sugar apple	*Annona squamosa* L.	Annonaceae	Fruits	E, N	+++
Cocoa	*Theobroma cacao* L.	Malvaceae	Plantation	E, N	+++
Peepal	*Ficus religiosa* L.	Moraceae	Ornamental	E, N	++
Coconut	*Cocos nucifera* L.	Arecaceae	Plantation	E, N, A	+++
Teak	*Tectona grandis* L.	Lamiaceae	Timber	E, N, A	-
Banana	*Musa paradisiaca* L	Musaceae	Fruits	E, N, A	++
Guava	*Psidium guajava* L.	Myrtaceae	Fruit	E, N, A	-
Maize	*Zea mays* L.	Poaceae	Millets	E, N, A	+
Spanish Cherry	*Mimusops elengi* L.	Sapotaceae	Ornamental	E, N, A	-

E - Hosts supporting up to egg stage; E,N – Hosts supporting both egg and nymphal stages, E,N- Hosts supporting egg, nymphal and adult stages; + denotes – presence (number of + denotes their relative abundance); - denotes absence

Fortunately, only in six host plants, the RSW was able to complete its life stage and successfully emerge as adults viz., coconut, teak, banana, guava, maize and Spanish Cherry. Among them coconut & banana house the parasitoid in larger numbers and aid in natural suppression of the pest. The hosts viz., *Annona muricata, A. reticulata, A. squamosa, Theobroma cacao* and *Ficus religiosa,* which also housed parasitoids in larger numbers should be viewed in a positive perspective in that, these hosts could be used as banker crops in and around coconut plantations so that the emerging parasitoids will aid in biosuppression of the target pest. The concept of banker plants has been suggested by Gurr et al. (2017) who suggests that banker plants provide shelter and serve as a source habitat and attract parasitoids and predators in the main crop so as to suppress the target pest population. The successful parasitism by *E. guadelopae* in coconut and banana deserves special mention as management of the invasive RSW through any other mean in these crops will be extremely difficult. Five host plants, *viz., Annona muricata, A. reticulata, A. squamosa, Theobroma cacao* and *Ficus religiosa* supported the growth and development up to nymphal stages only and in all these hosts, the aphelinid parasitoid, *Encarsia guadeloupae* were found in significant numbers. These hosts may serve as eco-feast crops / banker plants and could be utilized as border/ bund crops. Once established these plants will serve as 'parasitoid banks' and help in biosuppression of RSW in the main crop viz., coconut.

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AUTHOR CONTRIBUTION STATEMENT

D. Nandhini: Executed survey, documented host plants and helped documenting draft.

T. Srinivasan: conceived idea, designed research, assisted identification of host plants and drafted the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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POPULATION DYNAMICS OF MAJOR INSECT PESTS OF CUCUMBER

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ABSTRACT

This study on incidence of the jassid *Amrasca biguttula biguttula* in cucumber reveal that the incidence begins from 10th standard meteorological week (SMW) (0.56 jassid/ three leaves) with a peak (2.96 jassids/ three leaves) in the 16th SMW. Whitefly *Bemisia tabaci* appeared during 11th SMW (0.80 whiteflies/ three leaves), increasing to a peak (2.65 whiteflies/ three leaves) during 17th SMW. Fruit fly *Bactrocera cucurbitae* infestation on fruit began from the 13th SMW (16.35%), with a maximum (45.03%) during the 17th SMW. Leaf miner *Liriomyza trifolii* infestation started from the 10th SMW (0.66%), with leaf damage being maximum (4.45%) at the 15th SMW. Maximum and minimum temperature showed significant and positive correlation with *B. cucurbitae* infestation, whereas the morning and evening relative humidity had non-significant and positive association.

Key words: *Amrasca biguttula biguttula Bactrocera cucurbitae*, *Liriomyza trifolii*, *Bemisia tabaci*, cucumber, population dynamics, correlation coefficients, peak incidence, temperature, relative humidity

Cucurbits constitute the largest group of summer vegetables, and in India, it occupies an area of about 82 thousand ha with the production of 1260 thousand mt during 2018 (Anonymous, 2018). Cucumber is one of these, and like other cucurbits, it is infested by a number of insect pests right from the germination up to harvesting stage, but a few of these viz. red pumpkin beetle *Raphidopalpa foveicollis*, fruit flies *Bactrocera cucurbitae* and hadda beetle *Epilachna dodecasigma* are of serious concern (Gupta, 2004). *B. cucurbitae* and *R. foveicollis* caused 100% fruit damage mostly in the pumpkin, cucumber and bitter gourd (Jyoti and Rajbhandari, 2015). Yield losses in % due to *B. cucurbitae* in different cucurbits crops is 20-39% in cucumber, 60-80% in bitter gourd, 63% in ivy gourd and snake gourd and 50% in sponge gourd. Apart from causing the direct damage, many of them also act as vectors for several viral diseases. This study explores the population dynamics of *Amrasca biguttula biguttula Bactrocera cucurbitae*, *Liriomyza trifolii*, *Bemisia tabaci* on cucumber.

MATERIALS AND METHODS

Field experiment was conducted during summer 2018 at the Horticultural Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The variety Gujarat Cucumber 1 was sown in non-replicated plot design in 12 x 6 m and spacing 120 x 60 cm, with transplanting done on 13.7.2018 and last harvesting, being in fourth week of November, 2018. Ten plants were randomly selected and tagged for recording the observations. Weekly observations were made from one week after sowing till harvest. The number of jassid and whitefly counts was carried out on three leaves (proximal, middle and distal). Leaf miner and fruit fly incidence was recorded on % infestation basis. The data were statistically analyzed using the SPSS software and results cross checked using OPSTAT software.

RESULTS AND DISCUSSION

The results depicted in Table 1 and Fig. 1 reveal the population dynamics/ incidence of jassid, whitefly, leaf miner and fruit fly correlate with weather parameters viz., temperature, relative humidity, wind velocity, bright sunshine. *Amrasca biguttula biguttula* incidence started from 10th SMW (0.56 jassid/ three leaves) and reached its peak (2.96 jassids/ three leaves) in the 16th SMW. The correlation coefficient revealed that incidence was positively and non-significantly correlated with morning

Table 1. Correlation between pest incidence and weather parameters (Summer, 2018)

Pests	Weather parameter					
	Temperature (°C)		Relative humidity (%)		Wind velocity (km/ hr)	Bright sunshine (hr/ day)
	Maximum	Minimum	Morning	Evening		
Jassid	0.205	0.142	0.210	0.005	-0.042	0.064
Whitefly	0.490	0.487	0.477	0.363	0.213	0.175
Fruit fly damage (%)	0.705**	0.655*	0.535	0.488	0.355	0.321
Leaf miner damage (%)	0.156	-0.065	-0.042	-0.331	-0.205	0.061

*Significant at p = 0.05; **p = 0.01

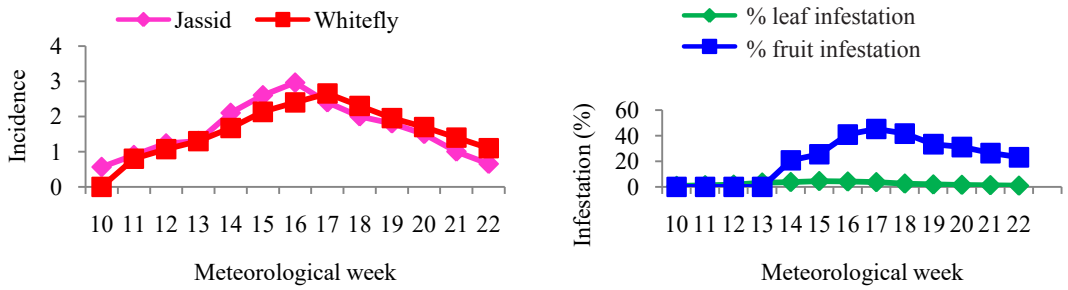


Fig. 1. Incidence of pests on cucumber

and evening relative humidity RH (r = 0.210 and 0.005) and maximum and minimum temperature (r = 0.205 and 0.142). Purohit (2010) observed that the incidence of *A. biguttula biguttula* commenced during 38th SMW and the peak was during second week of October in cucumber. The incidence of *B. tabaci* started from 11th SMW (0.80 whitefly/ three leaves), reached its peak (2.65 whiteflies/ three leaves) in 17th SMW; correlation revealed a non-significant and positive correlation with maximum and minimum temperature (r = 0.490 and 0.487) and morning and evening RH (r = 0.477 and 0.363). Purohit (2010) observed that *B. tabaci* initiated during 38th SMW and the peak was during third week of October at Udaipur. Saha et al. (2018) recorded peak incidence of *B. tabaci* during the last week of May to second week of June. Similarly, positive correlation of *B. tabaci* incidence with maximum and minimum temperature, and negative correlation with maximum and minimum RH had been eported by Sunil (2015); a significant and positive correlation with maximum and minimum temperature was observed by Selvaraj and Ramesh (2012) and Saha et al. (2018); and it was a significant and negative correlation with mean RH (Purohit, 2010), and with evening RH (Selvaraj and Ramesh, 2012). It was a non-significant and positive correlation with mean temperature (Purohit, 2010), and negative and non-significant with morning RH (Selvaraj and Ramesh, 2012 and Saha et al., 2018).

The incidence of *L. trifolii* began during 11th SMW (1.20%), reached its peak (4.45%) in 15th SMW; it was observed from second week of March to last week May; its incidence when correlated with weather factors revealed that the morning and evening RH (r = -0.042, -0.331) and minimum temperature (r = -0.065) have a non-significant and negative relationship, but maximum temperature had anon-significant and positive correlation (r = 0.156). Variya and Bhut (2014) observed that leaf miner damage on leaves was noticed soon after the 1st week of transplanting (40th SMW) and remained up to 3rd week of January (3rd SMW) in tomato. Saha et al. (2018) observed a significant and positive correlation with maximum and minimum temperature, and a non-significant and negative one with RH. About *B.cucurbitae*, incidence in fruits commenced after four weeks of sowing, and ranged from 16.35 (13th SMW) to 23.03% (22nd SMW), with peak being 45.03% at 17th SMW; and there existed a positive and highly significant correlation with maximum temperature (r = 0.705**), and with minimum temperature (r = 0.655*). These results are in close agreement with the observations of Pareek and Kavadia (1986) on bitter gourd. Its maximum infestation was up to 45.03%, agreeing with that of Kumar (2001) and with those of Sunil (2015) in bitter gourd.

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CONFLICT OF INTEREST

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OCCURRENCE OF CERAMBYCID BORER *BANDAR PASCOEI* (LANSBERGE) IN PUNJAB

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ABSTRACT

This study brings out the occurrence of cerambycid borer *Bandar pascoei*, (Lansberge) from mango growing regions of Punjab. The redescription of the species alongwith distribution records are also given.

Key words: Borer, *Bandar pascoei*, Coleoptera, Hoshiarpur, Punjab, mango growing regions, survey, light trap

Recently, cerambycid borers viz., *Dorysthenes (Lophosternus) huegelii* (Redtenbacher), *Aeolesthes holosericea* (Fabricius, 1787) and *Coptops aedificator* (Fabricius) have been reported on citrus and mango in Punjab, respectively causing significant damage to the plants (Singh and Sreedevi 2018; Singh and Sreedevi 2019; Singh et al. 2020). Stem borers of the Cerambycidae family are one of the serious pests of mango. Besides, *Batocera rufomaculata* (De Geer), *Glenea multiguttata* Guerin-Meneville has been reported on mango (Reddy et al. 2014). After hatching, neonate larva initially feeds under the bark and make tunnels (2-3 cm wide) through sapwood (Reddy et al. 2018). Keeping in view the increasing incidence of cerambycid borers in Punjab, surveys were aimed at studying the emerging cerambycid borer pests.

MATERIALS AND METHODS

The study was conducted during June-July from 2017 to 2021. Mango growing areas of the submountainous zone of Punjab (31°31'38.4780''N, 75°54'49.2228''E) were studied for diversity of Cerambycidae. The light traps (160 W) were installed to attract the beetles at night at MS Randhawa Fruit Research Station, Gangian, district Hoshiarpur, Punjab. These beetles were sent to ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India for identification.

RESULTS AND DISCUSSION

Identification done on morphological basis confirmed the 20 specimens as *Bandar pascoei* (Lansberge). The cerambycid was identified as *Bandar pascoei* (Lansberge, 1884) (Macrotomini: Priorinae: Cerambycidae) (Fig. 1). A diagnosis of this reveals the following:



Fig. 1. *Bandar pascoei*

Body dark brown, elytra pale brown towards apex, distal segments of the antennae and hind legs and tarsi more or less reddish Length 61 mm. Head punctured in front and between the eyes, elongated behind the eyes, antenniferous tubers closely placed. Antennae 11 jointed, moderately long reaching 3/4th of the elytra, first joint relatively short and the third segment longest, asperate at the base. Pronotum rugose with a central small patch without punctures, edges with numerous short spines. Elytra densely granulose, the granules stronger towards the base, especially near the suture. Metasternum bare of pubescence, with a sparsely punctured triangular area clearly marked off by a slightly raised line from the closely punctured part on each side. Front legs spinose beneath, the femora thickened, the tibiae elongated and slightly curved; the middle and hind legs much less spinose.

Distribution: Bhutan (Lazarev, 2019); Borneo, Indonesia (Sumatra, Java) (Kariyanna et al. 2017); Malaysia (Chung et al. 2013); Nepal to Southern China (Komiya, 2016); Philippines (Hudephol, 1987); Sri Lanka (Makihara et al. 2008); Tibet, Myanmar, Thailand (Komiya, 2016); South Laos, Vietnam (Cuong et al. 2017); Banga Island, Billiton Island, Borneo, Hainan Island, Taiwan, Japan, Calemandan (Lingafelter et al. 2013); India**:** Assam, Arunachal Pradesh, Himachal Pradesh, Sikkim, Tripura, West Bengal (Kumawat et al., 2015; Mitra et al., 2017; Kariyanna et al., 2018); Chhattisgarh and Madhya Pradesh (Majumder et al., 2014; Gajendra and Prasad, 2015; Kariyanna et al., 2017; Kariyanna et al., 2018); Gujarat (Kariyana et al., 2018).

Host plants: *Castanea mollisima, Diospyros kaki, Malus pumila, Pistacia chinensis, Prunus armeniaca, P. persica, Pyrus serotina* and *Quercus variabilis* (Gressitt 1951; Makihara et al. 2008).

Remarks: Quentin and Villiers (1981) considered *B. pascoei* to be synonymous with *B. khooi.* Hüdepohl (1987) reported this species from the Philippines. This is the first documentation of *B. pascoei* in Punjab.

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CONFLICT OF INTEREST

No conflict of interest.

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RESISTANCE TO MIRID BUG *CREONTIADES BISERATENSE* (DISTANT) IN COTTON

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ABSTRACT

A field experiment was carried out in the AICRP on Cotton, Haradanahalli Farm, Chamarajanagar, Karnataka to know the association of morphological characters of 15 cotton genotypes (different resistance category) influencing resistance to mirid bug, *Creontiades biseratense*. Cotton morphological parameters viz., trichome density on leaves, squares and midvein showed a significance difference with the genotypes and the higher incidence of mirid bug was recorded on genotypes having more trichome density. Similarly, genotypes having higher boll rind thickness and length of petiole recorded the higher incidence. of *C. biseratense.* There was a significant positive correlation between *C. biseratense* incidence and trichomes on leaves (r = 0.90), trichomes on squares (r = 0.89**), trichomes on midvein (r = 0.91**), boll rind thickness (0.80**) and length of petiole (r = 0.60*). However thickness of leaf showed the non-significant negative difference among the genotypes (r = -0.03).**

Key words: Cotton, resistance, incidence, screening, *Creontiades biseratense*, trichome density, midvein, boll rind, thickness of leaf, squares, length of petiole, correlation coefficient

Cotton is one of the most commercially grown fibre crop in the world, plays a pivotal role in economic and social affairs of the world. In India cotton is most important cash crop accounting for 65 % **(**CCI, 2021) of the textile industry's fibre and handloom cloth quality has been widely praised in the market for its fineness and comfort. It occupies a significant position in the Indian national economy as it provides livelihood to millions of people so it is one of the major foreign exchange earners of the country. India has the world's largest cotton cultivation area, accounting for around 41% of global cotton area and providing 21% of total cotton production, increasing from 12.50 million hectares to 13.00 million hectares. **(**CCI, 2021). Thus, India ranks first in cotton area and production by cultivating in an area of 132.85 lakh hectares with a production of 352.48 lakh bales and productivity of 451 kg/ ha during 2020-21 **(**CCI, 2021). Among the many constraints in cotton production, insects are becoming a major one. About 1326 species of insect pests are known to attack the cotton crop across the world at different growth stages of the crop. In recent days sucking pests are causing severe menace in cotton ecosystem (Hanchinal et al., 2009; Meghana et al., 2018). In South India, the mirid bug, *Creontiades biseratense* (Distant) was newly recorded and creating a major problem in *Bt* cotton that leading to significant yield loss in cotton cultivation (Patil et al., 2006). Among sucking pests, the mirid bugs are observed to be the serious pest of Bt cotton (Sahu and Samal, 2020) and their survey on incidence in Bt cotton carried out in Karnataka with varied level of climatic factors (Vinayaka and Nandihalli, 2019). Hence, identification of mirid bug tolerant and high yielding genotypes need to be identified. To develop the resistant or tolerate cultivar against the pest, studying the host plant resistance mechanism is crucial. Therefore present investigation of morphological association of resistance against mirid bug was conducted during 2020-21.

MATERIALS AND METHODS

To establish the resistance and susceptibility in relation to incidence of mirid bugs and major plant morphological characters in cotton, the observations on length of petiole, thickness of leaf and bolls, trichome density on leaves and square, density of trichome on midvein were made by adopting standard procedures by selecting 15 genotypes *viz.,* Br-24b-2676, Br-24b-2673, Br-24b-2675, Br-24b-2671, Br-2b-373, Br-2b-375, Br-24b-2678, CET H×B 20608, Br-2b-378, CET H×B 20609, CET H×B 20606, Br-13a-2663, CET H×B 20605, Br-2b-358 and Br-2b-359 representing different level of incidence at 60 DAS from screening trial of Kharif 2020.The observation on mirid bug was made from 10 squares/ plant from ten randomly selected plants. The mean data on the plant morphological

characters were worked out and subjected for ANOVA (Gomez and Gomez, 1984; Hosmand, 1988) and means were separated by Tukey's HSD (Tukey, 1965) for interpretation. Categorization of genotypic resistance of genotypes was made by following the procedure outlined by Croxton and Cowden (1964). Length of petiole was measured by using scale and thickness of leaf lamina and boll bract was measured by vernier caliper. Observations were taken from three leaves and bolls/ plant. Trichome density was measured on leaf lamina and square. The number of hairs on lamina, midvein and square were counted from upper side of leaf and square visually with the help of compound microscope at 10x resolution. Observations were taken from three leaves and square/ plant and three plants from each replication. Further, to study the relationship between the mirid bug infestation and plant morphological characters, the mean data was subjected to multiple linear regression (MLR) analysis techniques by fitting different functions.

RESULTS AND DISCUSSION

The trichome density of leaves among the different genotypes showed a significant difference. The susceptible and highly susceptible genotypes recorded the higher trichome density compared to moderately susceptible, moderately resistant and resistant genotypes. The lowest trichome density of 98.33, 99.33 and 100.33/ cm^2 was observed in Br-24b-2671(MR), Br-24b-2676(R), Br-24b-2678(MR) genotypes, respectively. These were on par with each other and showed a significant difference with other genotypes. This was followed by Br-24b-2673, Br-24b-2675, Br-2b-375 and Br-2b-373 which were on par with each other and reacted as moderately resistant and moderately susceptible. The highest trichome density was recorded in HS genotypes viz., CET H×B 20605 (173.67/ cm^2), Br-2b-358 (172.33/ cm^2) and Br-2b-359 (163.67/ cm^2) which were on par with each other. This was followed by Br-2b-378, CET H×B 20609, CET H×B 20606 and Br-13a-2663. The genotypes viz., Br-24b-2676(R) and Br-24b-2671(MR) recorded the lowest trichome density of 94 and 102/ cm^2 on squares, respectively. These two genotypes were on par with each other and recorded a significant difference with other genotypes. This was followed by Br-24b-2678 (109/cm^2), Br-24b-2673(119/ cm^2), which were on par with each other. Thus, resistant and moderately resistant group of genotypes showed lesser trichome density (Table 1).

Table 1. Incidence of mirid bug *C. biseratense* in relation to plant morphological characters (kharif 2020)

S.No.	Genotypes	Resistance category	Mirids /10 squares/ plant	Trichome density/cm^2 on			Thickness (mm)		Length of petiole (cm)
				Leaves	Squares	Midvein	Leaf	Boll rind	
1	Br-24b-2676	R	1.43	99.33^{a}	94.00^{a}	102.66^{a}	0.33^{ab}	1.40^{a}	5.63^{a}
2	Br-24b-2673	MR	2.10	113.67^{ab}	119.00^{cde}	116.00^{abc}	0.34^{ab}	1.45^{a}	6.03^{abc}
3	Br-24b-2675	MR	2.20	122.00^{bc}	126.66^{ef}	122.66^{bcd}	0.34^{ab}	1.62^{ab}	7.17^{abcd}
4	Br-24b-2671	MR	2.21	98.33^{a}	102.00^{ab}	107.00^{ab}	0.33^{ab}	1.54^{ab}	5.73^{ab}
5	Br-2b-373	MS	2.73	129.33^{bcd}	113.33^{cd}	134.33^{d}	0.31^{ab}	1.74^{ab}	8.69^{d}
6	Br-2b-375	MS	2.93	128.33^{bcd}	138.00^{g}	131.66^{cd}	0.29^{ab}	2.36^{ab}	8.50^{d}
7	Br-24b-2678	MS	3.00	100.33^{a}	109.00^{bc}	108.00^{ab}	0.31^{ab}	1.57ab	5.80^{ab}
8	CET H x B 20608	S	3.80	135.33^{cde}	122.66^{de}	138.66^{de}	0.26^{a}	1.99^{ab}	9.23^{d}
9	Br-2b-378	S	4.33	160.33^{fg}	152.66^{hi}	173.33^{fg}	0.31^{ab}	2.29^{ab}	8.20^{cd}
10	CET H x B 20609	S	4.53	152.33^{ef}	144.33^{gh}	186.33^{gh}	0.34^{ab}	1.88^{ab}	8.53^{d}
11	CET H x B 20606	S	4.60	147.33^{def}	142.33^{g}	153.33^{e}	0.25^{a}	2.10^{ab}	7.73^{abcd}
12	Br-13a-2663	HS	5.00	144.00^{def}	134.66^{fg}	151.33^{e}	0.40^{b}	2.02^{ab}	8.07^{bcd}
13	CET H x B 20605	HS	5.13	173.67^{g}	156.00^{i}	169.00^{f}	0.37^{ab}	2.53^{b}	8.47^{d}
14	Br-2b-358	HS	5.50	172.33^{g}	168.33^{j}	186.33^{gh}	0.27^{a}	2.25^{ab}	8.73^{d}
15	Br-2b-359	HS	6.21	163.67^{fg}	172.33^{j}	191.66^{h}	0.32^{ab}	2.31^{ab}	7.87^{abcd}
	SE m ±		-	4.18	1.28	2.32	0.02	0.20	0.47
	CD @ p=0.05		-	12.12	3.73	6.72	0.08	0.58	1.37
	Correlation coefficient			0.90^{**}	0.89^{**}	0.91^{**}	-0.30	0.80**	0.60^{*}

Values followed by common letters non-significant at p=0.05 as per Tukey's HSD, (Tukey, 1956); R- resistant, MR- moderately resistant, MS- moderately susceptible, S- susceptible, HS- highly susceptible

While, the maximum trichome density on square was observed on Br-2b-359 (172.33) this was on par with Br-2b-358 (168.33) and recorded a significant difference with other genotypes. Similarly, the lesser trichome density on midvein was observed on resistant and moderately resistant group of genotypes and the trichome density was varied from 102.66 to 122.66/ cm^2. This was followed by moderately susceptible genotypes which were on par with each other and showed a significant difference with susceptible and highly susceptible genotypes. While, the higher trichome density of 191.66/ cm^2 was observed in Br-2b-359 (HR), followed by Br-2b-358 (168.33/ cm^2). The higher incidence of mirid bug was recorded on genotypes which have more trichome density on leaves, squares and midvein probably, this influenced the egg laying behaviour of mirid bug. Hence, these genotypes recorded more incidence of mirid bug compared to less resistant and moderately resistant group of genotypes which have less trichome. The thickness of leaf varied from 0.25-0.40 mm among all genotypes. The lowest thickness of 0.25, 0.26 and 0.27 mm was recorded in CET H×B 20609(S), CET H×B 20606 (S) and Br-2b-358 (HS), respectively. Likewise, higher thickness was recorded in Br-13a-2663(0.40 mm) and CET H×B 20605 (0.37 mm) which also recorded the higher incidence of mirid bug. There was no much influence of leaf thickness on incidence. Whereas boll rind thick have positive association with mired bug incidence because as thickness increases mired bug population also increases. It was evidenced in genotypes CET H×B 20605 (2.53 mm), Br-2b-375 (2.30) and Br-2b-358 (2.25 mm), which were recorded more incidence of mirid bug (Table 1). Length of petiole varied from 5.63-9.23 cm among different genotypes. The genotypes viz*.,* Br-24b-2676(R) and Br-24b-2671(MR) recorded the least petiole length of 5.63 and 5.73 cm respectively. While, the resistant and moderately resistant group of genotypes showed lesser length of petiole as compared with other group of genotypes (Table 1). The genotypes with more petiole length were found to be susceptible and length varied from 7.73-9.23 cm. Maximum petiole length was recorded on CET H×B 20608 (9.23 cm) and was on par with CET H×B 20609 (8.53 cm) and CET H×B 20605 (8.47 cm) where these genotypes recorded a higher incidence of mirid bug.

The correlation studies between mirid bug incidence and morphological parameters revealed a significant-positive association with morphological characters *viz.,* trichome on leaves (r=0.90**), trichomes on square (r=0.89**), trichomes on midvein (r=0.91**) and these results are probably due to influencing of egg laying behaviour of mirid bug. While, thickness of boll rind (r=0.80**) and length of petiole (r=0.60*) also showed the significant positive relation with mirid bug incidence (Table 1). Hence, lesser trichomes density and lesser boll rind thickness characters are the promising parameters highlighted from this study to use in resistance breeding. This might be due to fact that the bolls were the most preferred part by both nymphs and adults of mirid bug.

The association of morphological components with the incidence of mirid bug was in close conformity with the earlier findings of Bariola (1969) and Benedict et al. (1983). They reported that the polyphagous mirid pest of cotton *viz.,* tarnished plant bug, *Lygus lineoralis* and western tarnished plant bug *Lygus hespereus* expressed a positive association of trichome density for incidence of mirid bug, and this might be due to the preference of higher trichome density for egg laying. Likewise, Chikkarugi and Balikai (2011) observed trichomes on the lower leaf surface of genotypes that are resistant to shoot fly in sorghum. Prakash et al. (2013) also reported more mirid bug incidence on genotypes which have more trichome density. Hence, the hairy varieties like NCS-145, MRC-7351 and Tulsi-144 were more prone to mirid attack as compared to glabrous varieties like Brahma, VICH-303 and RCH-530. But the rind thickness and leaf thickness were not significantly correlated with the incidence of mirid bug. These are in contrast to the findings of Deb et al. (2015), Gonde et al. (2015), Amin et al. (2016) who reported that the higher trichome density hinders the insect movement and activity on plant. Present results corroborate those of Khan et al. (2014) and partially of Murugesan and Kavitha (2010) and Rizwan et al. (2021). Harishkumar (2015) reported the significant positive relation of mirid bug incidence with trichome density on lamina, midvein and leaf thickness. In contrast, number of hairs on leaf was negatively associated but positively associated with bract size as reported by Shalini (2010).

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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MANAGEMENT OF RODENT PESTS IN WHEAT SOWN UNDER DIFFERENT TILLAGE SYSTEMS

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ABSTRACT

Investigation to manage rodent pests in wheat crop sown with different tillage methods was conducted. The results revealed maximum efficacy in the form of control success (%) on plain bait consumption and burrow count basis with conventional (92.30) followed by zero tillage (84.74) and maize residue tillage (74.38). In these, rodenticide treatments with double burrow baiting was followed by single paper baiting. Lesser % cut tillers (0.39-0.53) and damage 9.56-15.71 kg/ 0.4 ha were observed in these. Burrow count in maize residue and zero tillage was 2.15 and 1.60x more than conventional tillage. Thus, rodenticide treatments with double burrow baiting at vegetative stage in December (bromadialone followed by zinc phosphide at 15 days interval) along with paper baiting (bromadialone) at reproductive stage (end February to 1st week of March) can be recommended against rodents in wheat crop to get higher economic returns (Rs.452.36-556.81/ 0.4 ha).

Key words: Bromadialone, burrow baiting, cut tillers, maize residue, paper bait, rodents, rodenticide, tillage, wheat crop, zinc phosphide

Wheat is a major cereal crop and in Punjab, it is grown on an area of 35.20 lakh hectares with production of 176.20 lakh mt (Anonymous, 2021). Crop residues are important natural resources and their conservation has many advantages (Singh et al., 2000; Khaliq et al., 2013, 2015), and it can induce sustainability (Collins et al., 1992; Hussain et al., 2015). Incorporation of residues in soil and surface mulching are important tactics (Jensen, 1997; Sidhu and Beri, 2005). When wheat crop is sown with happy seeder machine having maize residues as mulch in field, it inhabits more rodents than conventional methods. Tillage is one of the major crop production operations and is an important contributor to the cost of production (Uri,1999). It is estimated that tillage and sowing almost consumes 25% of the total operational energy in wheat production (Singh et al., 2008). Zero tillage and happy seeder methods are environment friendly and reduce cost of cultivation (Filipovic et al., 2006). There is an urgent need to reduce the cost of cultivation and increase the productivity with adoption of conservation tillage practices (Sharma et al., 2002). Happy seeder technology which involves low production cost is an important tool for residue management, but this practice provides congenial environment for rodent survival and their multiplication. Along with straw management, there is need to manage rodent population in wheat crop sown with different tillage systems. This study is designed to determine the time and method of rodenticide bait application required to keep the rodent population under check in wheat crop sown with maize residue, zero and conventional tillage methods under Punjab conditions.

MATERIALS AND METHODS

The experiment was conducted at the village Ladhowal District, Ludhiana, Punjab (30.91°N,75.85°E). Wheat crop is sown here in November-December and harvested during April. Wheat crop was sown in different tillage systems (i) fields having maize residues as mulching material left in the field and crop was sown with happy seeder machine directly (ii) zero tillage where crop was sown directly with drill machine without any tillage (iii) conventional tillage where 3-4 tillage operations (tillers and suhaga) were practiced. To carry out experiments, two rodenticides were used, bromadialone (0.005%) which is a chronic and anti-coagulant poison and other zinc phosphide (2%) which is an acute poison. Both rodenticides were purchased from local market under trade names Ruban^R and Rantil^R, respectively. For the preparation of one kg poison bait, properly mixed cracked wheat grains (935 g), powdered sugar (20 g) and vegetable oil (20 g) and zinc phosphide (25 g) was added to this mixture. On this similar pattern, bromadialone based poison bait was prepared where the weight of bromadialone was

taken as 20 g and of wheat grains as 940 g, with other ingredients remaining same (Anonymous, 2015a).

For burrow baiting, all the burrows were closed with soil by spade or khurpa, a day before treatment in the evening and all the reopened burrows next day (called live burrow) were treated with rodenticide bait. About 10 g of rodenticide bait was taken in a loose paper boat and placed about 15 cm deep inside each live burrow with a stick and again covered with soil. Similarly, for paper baiting, 10 g of rodenticide bait (@400g/ 0.4 ha) was placed on a piece of paper at 40 bait points in a 10x 10m grid in fields and near bunds of 0.4 ha each. To observe effect of rodenticides, two methods were used: pre- and post-census on plain bait consumption basis (without rodenticide) recorded before and after each treatment to calculate % control success (on plain bait consumption basis); and burrow count (burrow census) in which all the burrows were counted with naked eyes to record population and predominance of rodent species and to calculate % control success (on burrow count basis). Rodent damage (% cut tillers) was assessed at preharvest stage by taking five samples of 1 m^2/ 0.4 ha in two diagonal lines to cover center as well as all the four geographical sides. In each sample, the number of healthy tillers and tillers cut by rodents were counted. Yield loss (kg/ 0.4 ha) was calculated following Singla and Babbar (2010). There were five blocks selected, each of 0.4 ha, with three replications. In block I, double burrow baiting treatment during vegetative stage, 1st with bromadialone and 2nd with zinc phosphide (15 days after 1st treatment) along with single paper baiting with bromadialone at reproductive stage were practiced. In block II, single burrow baiting treatment with bromadialone along with single paper baiting with bromadialone were practiced. In block III only single paper baiting with bromadialone and in block IV single burrow baiting with bromadialone were practiced, whereas block V was kept as control, where no treatment was done. Burrow baiting was done during vegetative stage of wheat crop (7 days after sowing during December) and paper baiting during reproductive stage of wheat crop (end February to 1st week of March). In all selected blocks, farmer had grown recommended PBW-343 wheat variety and also adopted agronomic practices (irrigation, fertilizers, weed and insect control etc.) as recommended in "Packages of practices for rabi crops of Punjab" by Punjab Agricultural University, Ludhiana (Anonymous, 2015b). Data was calculated as mean± standered error and further two-way ANOVA and Tukey's HSD tests were performed to test level of significance.

RESULTS AND DISCUSSION

The results revealed that all the treatments show significant results, and after 1st treatment of burrow baiting with bromadialone the % control success (on burrow count basis) ranged from 51.30-61.16; in block I where 2nd burrow baiting was practiced with zinc phosphide at 15 days interval, higher control success was observed (76.93-82.07%) (Table 1). After 3rd treatment of bromadialone as paper baiting during reproductive stage in the end of February, maximum control success was in blocks having wheat sown with conventional tillage method followed by zero tillage and maize residue tillage; with maximum in block I (74.38-92.30%). Interestingly, similar results were observed when % control success was calculated on plain bait consumption basis; maximum control success was recorded in block I with conventional tillage (82.81) followed by zero tillage (78.73) and maize residue tillage (73.71); in block II, maximum ontrol success (71.26%) was recorded in conventional tillage, and in block III and IV where only single burrow baiting and single paper baiting were practiced, respectively, control success was less. Maximum rebuildup of rodent population was observed in controls and block III and IV. Conrol success (%) in all the treated blocks were non-significantly different with p=0.12 (at two way ANOVA) when compared among maize residue tillage, zero tillage and conventional tillage. Among all the treated blocks, block I was significantly different (p=0.00 and Tukey's HSD test).

No, significant difference was found in fields where single burrow/ paper baiting treatments were practiced. Interestingly, lower % cut tillers were recorded in treated block I (0.39-0.53) being lowest in conventional tillage. Similar pattern was observed as regards yield loss, with lesser loss being in block I and higher in block I. These parameters were lowest in conventional tillage. Wheat crop sown with happy seeder on maize residue has lower cost of cultivation and increases soil health. But it slightly encourages rodents due to leaves and cobs lying on the fields. In zero tillage, there is again least disturbance of soil which leads to higher rodent burrow count, as compared to conventional one. In all tillage methods, there was predominance of Indian gerbil *Tatera indica*, field mouse *Mus booduga* and lesser bandicoot rat *Bandicota bengalensis* (Table 2). In fields with maize residue and zero tillage, the number of burrows was 2.15 and 1.60x more than conventional tillage, respectively, and more so with maize residue tillage fields. Number of burrows in all the treated blocks were significantly different (p=0.00) when individually compared among maize residue, zero and conventional tillage. Also, there

Table 1. Efficacy off rodenticide treatments in wheat crop sown with different tillage methods

Block	Maize residue tillage				Zero tillage				Conventional tillage			
	% Control success (on burrow count basis)			% Control success (on plain bait consumption basis)	% Control success (on burrow count basis)			% Control success (on plain bait consumption basis)	% Control success (on burrow count basis)			% Control success (on plain bait consumption basis)
	After 1st treatment (BB)	After 2nd treatment (BB)	After 3rd treatment (PB)	After 3rd treatment (PB)	After 1st treatment (BB)	After 2nd treatment (BB)	After 3rd treatment (PB)	After 3rd treatment (PB)	After 1st treatment (BB)	After 2nd treatment (BB)	After 3rd treatment (PB)	After 3rd treatment (PB)
Block I	54.88± 2.51	76.93± 2.87	[a]74.38± 3.57	[a]73.71± 2.96	57.2± 2.01	77.97± 2.87	[a]84.74± 2.39	[a]78.73± 3.14	51.30± 1.01	82.07± 0.95	[a]92.30± 3.25	[a]82.81± 3.07
Block I	58.76± 2.38	41.28± 3.45	[b]67.64± 4.09	[b]63.04± 5.24	60.63± 1.84	51.54± 1.98	[b]72.72± 4.50	[b]66.02± 4.15	55.55± 0.75	40.77± 1.08	[b]88.88± 3.18	[b]71.26± 5.10
Block III	--	--	[b]60.55± 4.84	[b]56.63± 1.38	-	-	[b]62.50± 3.81	[b]57.89± 4.15	--	--	[b]68.75± 4.53	[b]61.46± 5.38
Block IV	59.81± 4.43	43.36± 3.10	[b]44.86± 5.32	[c]46.89± 2.49	59.39± 1.96	51.57± 3.54	[c]48.42± 3.57	[c]43.31± 2.65	61.16± 1.10	48.33± 3.76	[c]47.25± 1.24	[c]51.11± 3.20
Block V (Control)	--	--	--	--	-	-	--	--	--	--	--	--

BB=Burrow baiting; PB= Paper baiting; values mean± S.E.; a, b, c, shows significant difference between values along the column

Table 2. Burrow count, yield loss and cut tillers (%) during pre-harvest stage with rodenticide treatments in wheat crop sown with different tillage methods

Block	*Bandicota bengalensis*	*Muss booduga*	*Tatera indica*	Total	Yield loss (kg/ 0.4 hectare)	Cut tillers (%)
Maize residue tillage						
Block I	0.33± 0.02	3.33± 0.98	2.33± 0.27	[a]6.0± 1.24	[a]15.71± 1.25	[a]0.53± 0.07
Block II	4.0± 0.34	0± 0	3.0± 0.47	[a]7.0± 0.40	[a]21.3± 3.81	[a]1.22± 0.013
Block III	2.66± 0.44	4.0± 1.70	4.66± 0.27	[b]11.33± 2.68	[a]23.05± 4.71	[b]1.48± 0.12
Block IV	4.66± 0.72	3.0± 1.25	7.0± 2.05	[b]13.66± 3.92	[b]33.21± 3.06	[b]2.03± 0.50
Block V (Control)	5.66± 1.23	9.33± 1.44	8.66± 0.72	[c]23.66± 3.93	[c]46.13± 2.94	[c]4.65± 0.28
Zero tillage						
Block I	0.66± 0.27	0.66± 0.54	1.66± 0.27	[a]3.0± 0.47	[a]11.03± 1.73	[a]0.44± 0.13
Block II	1.0± 0.10	2.0± 0.14	0± 0	[a]3.0± 0.17	[a]16.10± 2.16	[a]0.74± 0.07
Block III	2.66± 0.98	1.66± 0.27	1.66± 0.72	[b]6.0± 1.25	[a]19.11± 3.14	[a]0.86± 0.08
Block IV	3.66± 0.54	2.0± 0.94	5.33± 0.98	[b]11.0± 1.24	[b]26.12± 2.76	[b]1.62± 0.12
Block V (Control)	4.0± 1.41	6.0± 1.89	7.33± 2.23	[c]17.33± 3.92	[c]37.6± 2.91	[c]2.85± 0.16
Conventional tillage						
Block I	0.0± 0.0	0.33± 0.27	0.66± 0.54	[a]1.0± 0.47	[a]9.56± 2.75	[a]0.39± 0.06
Block II	0± 0	1.0± 0.16	0± 0	[a]1.0± 0.16	[a]11.68± 3.48	[a]0.62± 0.11
Block III	1.33± 0.72	0.33± 0.27	3.33± 0.72	[b]5.0± 0.82	[a]18.41± 5.09	[a]0.75± 0.07
Block IV	1.33± 0.27	0.0± 0.0	3.66± 1.19	[b]5.0± 1.25	[b]24.11± 3.12	[b]1.58± 0.28
Block V (Control)	3.33± 1.09	1.33± 0.20	6.33± 0.72	[c]11.00± 1.63	[c]34.12± 4.02	[c]2.55± 0.46

Values mean± S.E.; a, b, c, shows significant difference between values along the column

was significant difference in burrow number between all treated blocks belonging to three tillage methods. Yield loss saved among treated blocks ranged from 10.01-30.42 kg/ 0.4 ha, being maximum in block I (Table 3). So, by adopting rodenticide treatment as suggested in block I, higher net benefit of Rs. 452.36-556.81/0.4 ha can be accomplished (Table 3).

In a study by Kocher and Kaur (2007) in sugarcane fields, double poison baiting with zinc phosphide and bromadialone @ 1600g poison bait/ ha at an interval of 15 days during end November gave significant reduction in grain loss. Singla and Babbar (2012) in sugarcane with three poison bait applications, first in July, second in October-November and third in January obtained good results. Bromadialone causes no bait shyness in rodents (Anonymous, 2015b). In a study, Singh et al. (2017) recorded higher control success in direct seeded basmati rice where burrow baiting was practiced with zinc phosphide at vegetative phase and paper baiting with bromadialone at reproductive phase. In potato, Kaur et al. (2018) obtained higher control success, lower cut tillers and yield loss, and post-harvest burrow count. Singla and Babbar (2010) observed about 4.84% rodent damage with yield loss of 196.0 kg/ ha among 29 villages from ten districts in Punjab. In conventional tillage 60-70% control success can be achieved when only single paper baiting practiced at reproductive phase. In zero tillage and maize residue tillage methods, there is least disturbance of soil which proliferate rodent population. Thus, it can be concluded that rodenticide treatments with double burrow baiting at vegetative stage during December (1st with bromadialone and after 15 days 2nd with zinc phosphide) along with paper baiting (bromadialone) at reproductive stage (end February to 1st week of March) are must to manage rodent pests in wheat crop sown with conventional tillage, zero tillage and maize residue tillage methods.

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Table 3. Economics of rodenticide treatments in wheat crop sown with different tillage methods

Block	Maize residue tillage				Zero tillage				Conventional tillage			
	Yield loss saved (kg/ 0.4 ha)	*Cost of saved wheat (Rs./ 0.4 ha)	Cost of rodenticide	**Net benefit (Rs./ 0.4 ha)	Yield loss saved (kg/ 0.4 ha)	*Cost of saved wheat (Rs./ 0.4 ha)	Cost of rodenticide	**Net benefit (Rs./ 0.4 ha)	Yield loss saved (kg/ 0.4 ha)	*Cost of saved wheat (Rs./ 0.4 ha)	Cost of rodenticide	**Net benefit (Rs./ 0.4 ha)
Block I	30.42	600.79	43.98	556.81	26.57	524.75	38.78	485.97	24.56	485.06	32.70	452.36
Block II	24.83	490.39	34.86	455. 53	21.50	424. 62	28.70	395.92	22.44	443.19	27.58	415. 61
Block III	23.08	455.83	22.54	433.29	18.49	365.17	22.54	342. 63	15.71	310.27	22.54	287.73
Block IV	12.92	255.17	20.16	235.01	11.48	226.73	12.32	214.41	10.01	197.69	10.08	187.61
Block V Control)		-	-		-	-	-	--	-	-	-	-

*Price of wheat crop (Rs. 1975/quintal) as per minimum support price by Government of India,2020-21; ** After deducting the cost of preparation of poison bait (zinc phosphide bait=Rs. 36.10/ kg and bromadiolone bait=Rs. 56.35/ kg)

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INCIDENCE OF MIRID BUG SPECIES COMPLEX IN BT AND NON-BT COTTON GENOTYPES

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ABSTRACT

The present study on the incidence of mirid bugs in relation to weather factors in Bt and non Bt cotton hybrids was carried out at the KVK, Haradanahalli, Chamarajanagar. During the study three species of mirid bugs viz., *Creontiades biseratense* (Distant), *Helopeltis theivora* (Waterhouse) and *Taylorilygus apicalis* (Fiber) were observed, of which *C. biseratense* was the dominant. Maximum incidence of *C. biseratense* (6.56/ 10 squares/ plant), *Helopeltis theivora* (3.60/ 10 squares/ plant) and *Taylorilygus apicalis* (2.40/ 10 squares/ plant) was observed at 42nd, 45th, 43rd standard meteorological weeks. Correlation coefficients revealed that maximum temperature has a significant negative association with incidence. However, non-significant negative association was exhibited by evening relative humidity (RH) and rainfall. In contrast to this, significant positive association was observed with morning RH, a non-significant positive one with minimum temperature.

Key words: *Creontiades biseratense, Helopeltis theivora, Taylorilygus apicalis*, Bt and non Bt cotton, population dynamics, correlation coefficients, relative humidity, temperature, rainfall

Cotton (*Gossypium* spp.) is an important fibre crop and India ranks first in cotton area and production with a productivity of 451 kg/ ha (CCI, 2021). After the wide scale adoption of Bt cotton in India from 2002 the area under Bt cotton increased to 95% and the sucking pests have attained a major pest status (Hanchinal et al., 2009; Anon., 2020). The mirid bug *Creontiades biseratense* (Distant) was first recorded in 2005 in Karnataka (Patil et al., 2006), later it was noticed in other states of south India viz., Tamil Nadu, Andhra Pradesh and Maharashtra. (Surulivelu and Dhara Jothi, 2007). In South India, mirid bug, *C. biseratense* is creating a major menace in Bt cotton, that leading to significant yield loss. Three species of mirid bugs (Miridae: Hemiptera) viz., *Creontiades biseratense* (Distant), *Compylomma livida* (Reuter) and *Hyalopeplus linefer* (Walker) are infesting cotton since 2005; of these *C.biseratense* is the most widespread as key pest particularly in Karnataka (Udikeri et al, 2011). *Helopeltis theivora* Waterhouse has been reported first time infesting cotton (Dharajothi et al, 2018). Both adults and nymphs damages developing flower buds/ squares and tender bolls. One to two days old bolls with dried petals intact provide a good habitat to the insects for feeding and sheltering. The characteristic symptoms of feeding on the flower bud shows oozing out of yellow fluid from the buds and staining of this yellow fluid on the inner surface of the bracts. Infested tender bolls have number of black patches on all sides of the outer surface of boll rind. It was observed shedding of most of the damaged squares and tender bolls happens and damage causes loss of seed cotton yield to the tune of 11.69% (Rohini et al., 2009; Vinaykumar, 2013). Majority of cotton growers are unaware of the mirid bugs and their damage, and population dynamics of these in Bt cotton is not known. As this causes economic damage this study explores the species of mirid bugs and their population dynamics in Bt and non Bt cotton.

MATERIALS AND METHODS

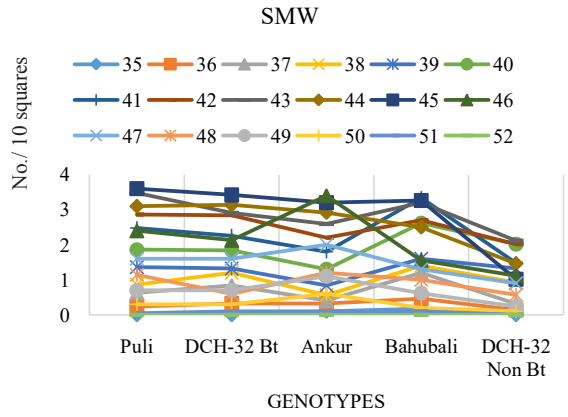
A field experiment was conducted at Haradanahalli, Chamarajanagara during 2020-21. The popular cotton cultivars Ankur (BG II) obtained from Ankur Seeds Pvt. Ltd, Puli (BG II), Shri Ram Bioseed Cotton Seeds, Bahubali (BG II) from Mahyco Company, and DCH-32 (Bt and non Bt) from University of Agricultural Sciences, Dharwad were sown in kharif 2020. The experiment was replicated thrice with randomized block design with a spacing of 90x 60 cm between rows and plants, respectively. All recommended agronomic practices were followed except insecticidal sprays (Anon., 2018). Such three blocks of a plot size 12x 9 m were maintained to evaluate the population dynamics. The observation on the incidence from flowering (40 DAS) and boll setting (60 DAS) stage

was recorded on weekly intervals till harvest. In each observation, ten plants were randomly selected and in each plant ten squares and bolls were examined during the evening hours. The data on incidence in each block was recorded plant-wise and mean counts/ plant was worked out. The species of mirids collected were got identified by the project of "Niche area of excellence on taxonomy of insects and mites", UAS, Bangalore. The incidence data were correlated with weather factors (of previous week) viz., maximum and minimum temperature, morning and afternoon relative humidity (RH) and total rainfall. The weather data were obtained from the agrometeorological observatory unit, Krishi Vignan Kendra, Haradanahalli, Chamarajanagara. The mean incidence at weekly intervals were subjected to Pearson's rank correlation analysis (Pearson, 1948).

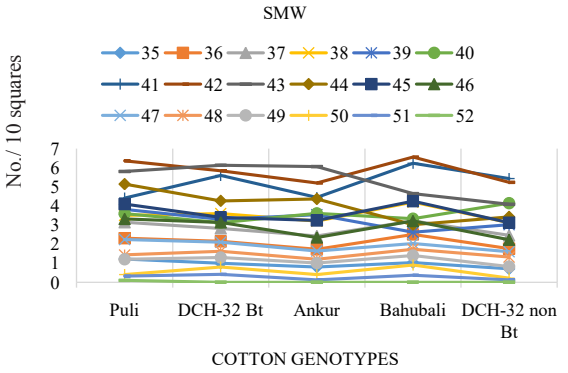
RESULTS AND DISCUSSION

The present study revealed the occurrence of three species of mirid bugs viz., Indian cotton mirid bug *Creontiades* (=*Poppiocapsidea*) *biseratense* (Distant), tea mosquito bug *Helopeltis theivora* Waterhouse and broken-backed bug *Taylorilygus apicalis* Fieber. Among these *C. biseratense* was the most common and dominant. The population dynamics of *C. biseratense* was analysed on different Bt cotton hybrids viz., Puli, DCH 32, Ankur, Bahubali and DCH-32 (non *Bt*) from 40 days after sowing and continued till harvest. The incidence was observed from 33rd standard meteorological week (SMW) of August, with gradual increase from first fortnight of September reaching a peak during second and third week of October. In Puli and Bahubali peak incidence was of 6.36 and 6.56 mirid bugs on 10 squares/ plant, respectively in 42nd SMW of October; it was at peak of 6.13 and 6.06/ 10 squares/ plant in DCH-32 (*Bt*) and Ankur, respectively in 43rd SMW of October; in DCH-32 non Bt cotton hybrid, incidence was 5.44/ 10 squares/ plant at 41st SMW (Fig. 1a). These findings agree with those of Manohar et al. (2012); Prakash et al. (2013) also reported similar incidence. In contrast, Vinaykumar et al. (2014) observed the maximum incidence during second fortnight of October (12.2 bugs/10 sqaures). Likewise, Jeer and Nandihalli (2019) also noticed the peak incidence of *C. biseratense* in 46th SMW.

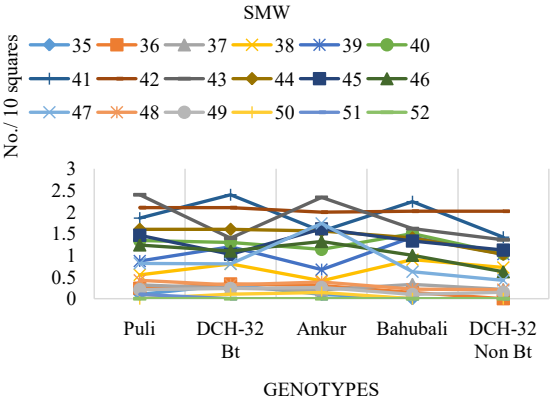
The population dynamics of *C. biseratense* revealed a significant positive association with morning RH (r = 0.51, 0.53, 0.56, 0.53 and 0.55) among all the hybrids *viz.,* Puli, DCH-32, Ankur, Bahubali and DCH-32 (non *Bt*), respectively. Likewise, minimum temperature also influenced revealed a positive significant association (Table 1). These results corroborate with those of Saravanan et al. (2017) who reported the significant negative correlation with maximum temperature and positive correlation with RH. Similarly, Naveen (2017) reported a significant negative one with maximum temperature (r = -0.76) and evening RH (r = -0.34) and significant positive one with minimum temperature (r = 0.88). Maximum incidence of *H. theivora* i.e.,3.60, 3.42, 3.40 and 3.34/10 squares/ on *Bt* hybrids Puli, DCH-32, Ankur and Bahubali, respectively was observed (Fig. 1b). These results conform with those



a. *Creontiades biseratense*



b. *Helopeltis theivora*



b. *Taylorilygus apicalis*

Fig. 1. Seasonal ncidence of mirid bugs on cotton genotypes

Table 1. Correlation coefficients- incidence of mirid bugs vs weather factors in cotton hybrids (kharif, 2020)

Hybrids	Mirid bug	T. Max (^{O}C)	T. Min (^{O}C)	Morning RH (%)	Evening RH (%)	Rainfall (mm)
Puli	C. b	-0.40*	0.38	0.51*	-0.27	-0.26
	H. t	-0.46*	0.08	0.60**	-0.35	0.28
	T. a	-0.43*	0.26	0.58**	-0.38	-0.33
DCH-32 (*Bt*)	C. b	-0.45*	0.41*	0.53**	-0.22	-0.27
	H. t	0.44*	0.10	0.64**	-0.35	-0.33
	T. a	-0.44*	0.35	0.52**	-0.36	-0.31
Ankur	C. b	-0.37	0.36	0.56**	-0.27	-0.32
	H. t	-0.46*	0.17	0.60**	-0.36	-0.24
	T. a	-0.46*	0.17	0.60**	-0.36	-0.24
Bahubali	C. b	-0.48*	0.42*	0.53**	-0.14	-0.24
	H. t	-0.42*	0.20	0.64**	-0.32	-0.34
	T. a	-0.39	0.30	0.57**	-0.38	-0.40*
DCH-32	*C. b*	-0.41*	0.40*	0.55**	-0.23	-0.31
(N*Bt*)	*H. t*	-0.35	0.22	0.59**	-0.34	-0.34
	T. a	-0.43*	0.33	0.58**	-0.35	-0.41*

C. b – Creontiades biseratense, H. t – Helopeltis theivora, T.a – Taylorilygus apicalis

of Roy et al. (2008); Roy et al. (2015) reported that *H. theivora* incidence was high during July to December and low during January-June. Incidence of *H. theivora* exhibited a significant positive association with morning RH (r = 0.60, 0.64, 0.54, 0.64, 0.55 and 0.59) among all the hybrids viz., Puli, DCH-32, Ankur, Bahubali and DCH-32 (non Bt), respectively; positive correlation with minimum temperature, except Ankur, which showed a slight negative association was also observed. Maximum temperature, evening RH and rainfall exerted a negative effect. While, Bt Cotton hybrids viz., Puli, DCH-32, Ankur and Bahubali exhibited a significant negative association with maximum temperature (Table 1). The present results are in close conformity with those of Roy et al. (2008) as regards minimum temperature and RH; Shaheen et al. (2015) also reported a positive correlation with mean temperature and humidity, but in contrast a significant positive association was there with rainfall.

The incidence of *T. apicalis* increased in September and reached maximum in October, it was 2.40, 2.40, 2.34 2.24 and 2.02/ plant on hybrids Puli, DCH-32, Ankur, Bahubali and DCH-32 (non Bt), respectively. (Fig. 1c). Similar observations were made by Malgvi et al. (2000), and Prajna (2018) reported it in last week of September and earlier week of October. Incidence was comparatively less compared to *C. biseratense* and *H. theivora*; and a significant positive association with morning RH (r = 0.58, 0.52, 0.60, 0.57 and 0.58) was observed in Puli, DCH-32, Ankur, Bahubali and DCH-32 (non Bt), respectively. Likewise, minimum temperature showed a non-significant influence on the population of *T. apicalis*. Maximum temperature exerted a significant negative association in Puli (r = -0.43), DCH-32 (-0.44), and Ankur (r = -0.46) Bt hybrids and DCH-32 (r = -0.43) non Bt hybrid. And, rainfall exhibited a significant negative association (r = -0.40) on Bahubali (Table 1). The present findings were in partial accordance with those of Ahirwal et al. (2009). Mishra et al. (2015) reported a significant positive association with temperature. Prajna (2018) reported that *Nesidiocoris tenuis* incidence had a positive association with maximum temperature and morning RH, while minimum temperature and evening RH influences the incidence negatively.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF CROP GROWTH STAGES ON THE FIELD POPULATION OF RICE HOPPERS

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ABSTRACT

The rice hoppers viz., brown plant hopper (BPH) *Nilaparvata lugens* (Stal), white-backed planthopper (WBPH) *Sogatella furcifera* (Horvath) and green leafhopper (GLH) *Nephotettix virescens* Distant cause loss in rice and the damage is influenced by plant growth stages. Field experiment has been conducted in this study to evaluate the effect of the growing stages on the incidence of these hoppers at the Agricultural Research Farm, Banaras Hindu University, Varanasi, Uttar Pradesh, India during kharif 2018 and 2019. The results revealed that the incidence is significantly varying among the growth stages, and positively correlated ($p < 0.05$). *Nilaparvata lugens* and *S. furcifera* are the most abundant during the maximum tillering to booting stage. In contrast that of *N. virescens* peaked during the booting to flowering stage.

Key words: Rice, *Nilaparvata lugens, Sogatella furcifera, Nephotettix virescens*, incidence, crop phenology, seedling, tillering, booting, flowering, ripening, population dynamics

Rice (*Oryza sativa* L.) is a crop belonging to the family Poaceae and is an internationally vital cereal crop and staple food (Sharma et al., 2018a; Ali et al., 2019). In the rice ecosystem, more than 250 insect pests and 350 beneficial arthropods had been reported from various agroecological zones (Ali et al., 2020). In 2018, around 20 insects were considered significant for the economic damage to the production of rice in India (Sharma et al., 2018b; 2019). Rice hoppers mainly brown planthopper (BPH) *Nilaparvata lugens* (Stl), white-backed planthoppers (WBPH), *Sogatella furcifera* (Horvath) and green leafhoppers (GLH), *Nephotettix virescens* Distant, are major rice pests that have severely harmed rice production in Asia (Sharma et al., 2019; Zhu et al., 2020). These rice hoppers cause direct damage to rice crops by sucking sap and plugging xylem and phloem with their sheaths during feeding period (Sharma et al., 2018c; Ma et al., 2021). Continued feeding of insects causes a drying process that results in "hopper burns". Planthoppers are vectors for viruses such as rice tungro, grassy stunt, and ragging stunt, as well as causing significant physiological damage to rice (Azgar and Yonzone, 2018). The insect pests' relationship with their host is an important factor in determining whether the insect develops and reproduces, as well as whether the populations grow (Awmack and Leather, 2002). Conversely, the plant's susceptibility to insect attacks is determined by its chemical constituents and morphological characteristics (Faruq et al., 2018). Thus, the selection of a stage-specific pest management strategy is an important step in pest management because the interactions between pest and host factors determine the level of infestation and crop stage of the species. The objectives of this paper were to examine the effect of crop growth stages on the population of rice hoppers during two consecutive kharif seasons during 2018 and 2019.

MATERIALS AND METHODS

The field experiment was carried out during kharif 2018 and 2019, at the Agricultural Research Farm, Banaras Hindu University, Varanasi, Uttar Pradesh, India (24°56'N,82°14' E, 82 masl). Variety (cv. Swarna sub-1) procured from the Agricultural Research Farm of the University and was sown in nursery beds on July 5th, 2018 and July 9th, 2019. Twenty-one day old seedlings were transplanted @ 2-3 seedlings/ hill on a plot size of 200 m^2. All the recommended agronomic practices were followed, and no insecticide was applied. The number of motile (adult and nymph) stages of BPH and WBPH was counted on five hills chosen at random in the experimental plot. Each hill was tilted twice or three times on the base to count the number of BPH and WBPH individuals as well as the hoppers that fell into the water. The GLH adults (male and female) were collected through sweep net with the sampling size of 5 sweeps/ replication at weekly intervals from 28-47

standard meteorological weeks (SMW). Tukey test ($p< 0.05$) was used to compare the abundance of hoppers with the crop growth stages (seedling, tilling, booting, flowering and maturing). The software IBM SPSS version 24.0 for Windows (IBM Corporation, Armonk, New York, USA) was used to perform the Tukey test.

RESULTS AND DISCUSSION

The phenological stages of rice plant significantly affected the *N. lugens* incidence; nymphs and adults were found active during the tillering to ripening stage (Table 1); pooled data revealed that their counts differed significantly with the growing stages ($F = 54.02$, df = 4, 15, $p < 0.05$). The booting stage (17.47± 2.22/ hill) of the crop was the most preferred, followed by the flowering (13.77± 1.54/ hill) and the tillering stages (8.76± 1.39/ hill); and nil during the seedling stage, while the peak was in the late tillering to flowering stage. The present findings agree with those of Hafizal and Idris (2013), who observed peaks during the vegetative stages. In contrast Skawsang et al. (2019) observed increase of incidence in the reproductive stage with a peak in early maturing stage. Faruq et al. (2018) observed its slightly higher abundance during the maximum tillering stage; its incidence increase during rice crop growth and then decline during preharvest phases when senescence begins to cause changes in nutrition, chemicals, and structures (Xu et al., 2013).

The incidence of *S. furcifera* was active during the late tillering to ripening stages, the pooled data revealed that it differed significantly with growing stages ($F = 65.16$, $df = 4, 15$, $p<0.05$), with booting stage showing maximum incidence (22.91± 2.71/ hill), followed by the tillering stage (15.42± 1.89/ hill); however, with the ripening stage it became less (7.12± 0.95/ hill), while it was nil in the seedling stage. Plant phenology exhibited a significant influence on the density of *S. furcifera*-its incidence increased in the vegetative stage and then declined as the harvest neared (Hafizal and Idris, 2013); the booting stage revealed maximum incidence followed by the maximum tillering and flowering stages (early maturing stage). The present results agree with those of Skawsang et al. (2019), Mangalgikar and Harsur (2012) and Xu et al., 2013). With *N. virescens* (nymph and adult), maximum counts/ hill was during the booting stage (10.33± 1.50/ hill) followed by the tillering stage (25.94± 2.08/ hill), with least counts in in the ripening stage (1.58± 0.07/ hill), nil in the seedling stage, and the peak observed in the late tillering to flowering stage. These findings agree with those of Zhong-xian et al. (2006). According to Xu et al. (2013), food availability increases during rice growth, and the population and density of leafhoppers peak during the booting stage. Similar observations were made by Hafizal and Idris (2013). In contrast, Faruq et al. (2018) observed maximum incidence during the seedling to early tillering stage. Thus, the crop growth stages had a significant impact on the incidence of rice hoppers.

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AUTHOR CONTRIBUTION STATEMENT

KRS: Conceptualization, Methodology, Investigation, Writing - original draft. SVSR: Supervision, Data curation, Writing - review & editing, Writing - original draft. SRB: Writing - review and editing. KS: Analyzed data.

CONFLICT OF INTEREST

No conflict of interest.

Table 1. Incidence of rice hoppers vs phenology of rice crop (pooled data, kharif 2018, 2019)

Phenological stage	Incidence (no./ hill)		
	Nilaparvata lugens	*Sogatella furcifera*	*Nephotettix virescens*
Seedling	0.00± 0.00^{d}	0.00± 0.00^{d}	0.00± 0.00^{c}
Tillering	8.76± 1.39^{c}	11.36± 1.64^{c}	5.59± 0.72^{b}
Booting	17.47± 2.22^{a}	22.91± 2.71^{a}	10.33± 1.50^{a}
Flowering	13.77± 1.54^{ab}	15.42± 1.89^{b}	7.67± 1.07^{b}
Ripening	5.05± 0.64^{c}	7.12± 0.95^{c}	1.58± 0.07^{c}
$F_{(4/15)}$	54.02	48.46	32.64

Mean (± SE) followed by the same alphabets in columns were not statistically different by the Tukey test ($p < 0.05$)

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ON FARM IMPLEMENTATION OF SUSTAINABLE IPM TECHNOLOGY AND ITS ECONOMIC ANALYSIS FOR BITTER GOURD INVOLVING FARMERS' CENTRED APPROACH IN HARYANA

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ABSTRACT

Area-wide field experiments were conducted in bitter gourd during 2014-2016 at Karnal, Haryana to study validation and economic viability and feasibility of adaptable and rational IPM technology involving farmers' driven holistic approach. The adoption of IPM technology, apart from lowering the incidence of major pests, reduced the number of chemical sprays to 5-9 from 15-17 in non-IPM fields with higher yields of 31.3, 22.3 and 18.75 mt/ ha in IPM compared to 29.5, 20.8 and 17.45 mt/ ha in non-IPM fields with marginally higher CBR of 1:1.80, 1:1.54 and 1:1.91 in IPM than 1:1.60, 1:1.30 and 1:1.68, respectively, in FP fields. There was a net income increase of Rs 23718.1, 35079 and 32403/ ha in IPM fields over FP fields. On an average, IPM farmers harnessed higher net return of Rs. 1.27 lakh/ ha with B:C ratio of 1:1.75 compared to the non-IPM farmers with net return of Rs. 0.97 lakh/ ha with a B:C ratio of 1:1.52.

Key words: Bitter gourd, IPM technology, thrips, whitefly, fruit fly, *Diaphania indica*, lady bird beetle, Cercospora leaf spot, begomovirus, natural enemies, spider, pesticides, prophylactic spray, net return, B: C ratio

Bitter gourd (*Momordica charantia* L.: Cucurbitaceae) is extensively cultivated throughout India, occupies an area of 0.09 m ha with a production of 1.05 mt (Anonymous, 2016)). India is still far behind many countries in terms of productivity owing to attack by several pests, of which fruit fly *Bactrocera cucurbitae*; thrips *Thrips palmi* and cucumber moth *Diaphania indica* and leaf curl (begomovirus) are important and cause substantial yield losses in Haryana. Quicker control strategy using indiscriminate and excessive reliance on chemical pesticides and quest of getting higher yields, has led to environmental destruction (Halder et al., 2010, 2013). It is not unusual for the bitter gourd growers to give upto 15 chemical sprays in a season, which most of the times are unnecessary without any appreciable increase in the yield. Information on development of IPM modules for the holistic management of pests in bitter gourd in a wider area approach is very scanty. Numerous management strategies for the pests of bitter gourd have been developed but these have mostly been dealt in isolation and have met with little desired success. The integration of all the pest management strategies in a farmers led approach/ mode could reduce application of harmful chemical pesticides to a great extent. Keeping this in view, validation of multifaceted adaptable IPM technology in bitter gourd was carried out in a participatory manner at farmers' fields to reduce the over dependence and reliance on chemical pesticides and protecting the ecosystem as a whole (Sardana et al., 2017).

MATERIALS AND METHODS

Three year trials on validation of IPM technology in bitter gourd crop were carried out during 2014-16 at Padhana, Karnal, Haryana. Before initiation of validation of IPM technology, adaptable IPM module for bitter gourd was synthesized based on the base line information, pest and natural enemies status in District Karnal from farmers; recommendations made by relevant research institutes and literature. The IPM module thus synthesized was validated during 2014 initially in an area of 10 ha acres comprising 25 farming families with the following interventions: seed treatment with *Trichoderma asperellum* @ 10 g/ kg seed, installation of cue lure traps (MAT) for fruit flies @ 25/ ha, raking of soil for exposing fruit fly pupae to sunlight and predatory fauna and dormant weeds, spray of fipronil 5SC against thrips, need based pray of *Bacillus thuringiesnsis* @ 2 g/ l against cucumber moth *Diaphania indica,* need based spraying of imidacloprid 17.8SL @ 40 g a.i.ha^{-1} for whitefly, hoppers, other sucking insects and prophylactic spray with thiophenate methyl 70WP @ 0.2% against *Cercospora* leaf

spot (*Cercospora citrulina*). The results on the pest incidence/natural enemies population and the economic viability of IPM were compared with non-IPM (farmers' own way of managing the pests) which consisted of only chemical pesticides which included mainly: imidacloprid 17.8SL @ 0.5 ml/ l, thiamethoxam 70 WG @ 0.5 g/ l, acetamiprid 20SP @ 0.33 g/ l, spinosad 45SC @ 0.5 ml/ l, cypermethrin 25EC @ 1 ml/ l, profenophos 40EC + cypermethrin 4EC @ 1 ml/ l, quinalphos 25 EC @ 2 ml/ l; dimethoate 30EC @ 2 ml/ l, mancozeb 75WP @ 2 g/ l, metalaxyl 8WP + mancozeb 64 WP @ 2.5 g/ l and streptocycline @ 0.02%. Local farmers often gave higher than recommended doses of pesticides and frequently applied unknown herbal tonic/micronutrient mixtures with different pesticides with apprehension to rejuvenate their crops. During 2015, the adaptable IPM technology was refined and revalidated on 20 ha comprising 50 farming families in the same location. Due to frequent fungal contamination, the installation of food bait traps and individual bagging of fruits was stopped due to difficulty in implementing. Use of *Bt* against cucumber moth was also discontinued as *Diaphania* did not appear to cause economic damage. During 2016, validated IPM technology was further refined and revalidated in 150 acres in Padhana which involved 75 farming families. Use of insecticides against fruit fly was discontinued as these were found ineffective.

Periodical observations were made on the major biotic fauna in bitter gourd at selected farmers' field, fruit flies trapped in cue lure traps, fruits damage due to melon fruit fly were recorded and percent fruit damage was woked out. For cucumber moth (larvae) population on square meter area (due to thick entangaled canopy) at five locations from each acre area was counted while thrips population were counted on one inflrescence from each plant and white fly on 10 leaves from each plant. For *Cercospora* leaf spot, 0-5 scale was used and based on this percent disease index (PDI) was calculated. For leaf curl, 5 spots were randomly selected from each field of one acre, and in each spot, 15 randomly selected growing tips of one feet length were inspected and % incidence was calculated. Leaf curl caused by begomovirus was confirmed using specific primers. For economic analysis, numbers of chemical sprays, cost of cultivation (per ha), yield (ton/ha), net returns (per ha) and cost :benefit ratio (CBR) were computed and results were presented in Table 2. To estimate the pesticide residue contamination, bitter gourd fruits (up to 50 g each) from both IPM and non-IPM fields were collected and analysed in pesticide residue lab of ICAR-IARI, New Delhi, India.

RESULTS AND DISCUSSION

The adoption of IPM technology resulted in reduction in incidence of all major pests in IPM plots compared to non-IPM plots; *D. indica,* which appeared from June onwards in early reproductive stage was effectively managed by a single spray of *Bt* @ 2 g/ l. In contrast, non-IPM farmers applied a series of chemicals which resulted in increasing the cost of production. Thrips incidence remained higher in non-IPM plots during 2014, 2015 and 2016 being 7.20, 6.90 and 4.3, respectively with an average of 6.10 whereas in the IPM plots these were only 4.8, 3.8, 1.9/ plant, respectively with an average of 3.5. Installation of cue lure traps @ 25/ ha for fruit fly management from flowering onwards and raking of soil resulted in significantly lower fruit damage i.e. 6.7, 4.5 and 2.3% during 2014, 2015 and 2016, respectively, as against higher fruit fly damage registred in FP fields i.e. 9.4, 9.5 and 9.5%, respectively during the same period. Effectivity of cuelure bottle traps for the management of fruit fly in cucurbits vegetable ecosystem has been well established (Sandeep Kumar et al., 2019; Anitha Kumari et al., 2021) (Table 1). Severity of *Cercospora* leaf spot ranged from 14.5-

Table 1. Pests and natural enemies scenario in IPM and non-IPM bitter gourd (2014-16)

Pest/ natural enemy	IPM fields				FP fields			
	2014	2015	2016	Average	2014	2015	2016	Average
D. indica/ sq m	1.2	0.8	2.0	1.3	4.2	3.0	5.6	4.2
Fruit fly (% damage)	6.7	4.5	2.3	4.5	9.4	9.5	9.5	9.5
Thrips (no./ inflorescense)	4.8	3.8	1.9	3.5	7.2	6.9	4.3	6.1
White fly (no/ 10 leaves)	2.0	2.9	4.3	3.1	6.4	5.1	11.6	6.0
Begomo virus (%)	29.8	21.6	35.7	29.0	42.5	34.5	47.7	41.5
Cecospora leaf spot (PDI)	16.6	14.5	34.9	22.0	22.0	17.9	48.7	29.5
Lady bird beetle/ sq m	0.4	2.5	1.8	1.5	0.2	1.0	0.8	0.7
Spiders/ sq m	1.0	4.6	2.5	2.7	0.4	2.0	1.2	1.2

Table 2. Economic analysis of IPM and non-IPM technologies in bitter gourd (2014-16)

Variables	2014		2015		2016	
	IPM	FP	IPM	FP	IPM	FP
Number of sprays	9.8	15.9	7.2	9.5 (17)	5.6	8.5 (15)
Cost of pesticides including labour cost (Rs/ ha)	15695	21725	12370	21467.5	9395	18590
Cost of cultivation (Rs.)	171545	177575	161395	172742.5	168320	178515
Yield (t/ ha)	31.30	29.5	22.32	20.18	18.74	17.45
Gross return / income *(Rs./ ha)	309992.5	295304.4	249505.7	225773.8	322522.2	300314.5
Net return (Rs./ ha)	138447.5	117729.4	88110	53031.3	154202.2	121799.5
Cost benefit ratio	1:1.80	1:1.60	1:1.54	1:1.30	1:1.91	1:1.68

*Sale price of bitter gourd Rs. 1000, Rs. 1118.8 and Rs. 1721/ q during 2014, 2015 and 2016, respectively.

34.9 PDI with an average of 22.0 PDI during the above period in farmers fields (FP) as against 17.9- 48.7 PDI with an average of 29.5 PDI in IPM fields. IPM fields suffered less from leaf curl i.e., 2014 (29.8%), 2015 (21.6%) and 2016 (35.7%) than the non-IPM fields. A similar observation was recorded by Sardana et al. (2012) while managing bell pepper through IPM. A large buildup of natural enemies, especially predatory spiders and coccinellids was observed in IPM fields. High population of spiders in IPM fields (1.0, 4.6 and 2.5/ sq m) than non-IPM fields (0.40, 2.0 and 1.2/sq m) was observed during 2014, 2015 and 2016, respectively (Table 1). Almost a similar trend was recorded with coccinellids. IPM technology, thus resulted in increased biodiversity. Sardana et al. (2012) and Sardana and Bhat (2016, 2017) from their studies concluded that IPM was safer to coccinellids and predatory spiders in pepper and onion ecosystems.

The mean fruit yields obtained were higher, i.e., 31.3, 22.32 and 18.74 t/ ha with an average of 24.1 t/ ha in IPM fields compared to farmer's practices (FP) where it was 29.5, 20.18 and 17.45 t/ ha during 2014, 2015 and 2016, respectively. The consumption of pesticides in terms of number of sprays had also come down gradually in IPM fields; while FP/non-IPM farmers continued to give higher sprays i.e 15.9, 9.5 (17) and 8.5 (15) in a season. During 2014, the cost of cultivation including plant protection measures was slightly higher in both IPM (Rs. 171545/ ha) as well as non-IPM (Rs. 177575/ ha) fields mainly due to purchase of staking materials viz., bamboo and iron/plastic wire that served for more than two years. During 2016, cost of various inputs including labour cost had increased and hence the cost of cultivation was marginally higher. From Table 2 it was evident that IPM adopted farmers had higher gross return of Rs. 3.09, 2.49 and 3.22 lakhs/ ha during 2014, 2015 and 2016, respectively, compared to Rs. 2.95, 2.25 and 3.0 lakh/ ha in case of non-IPM farmers. Same trend was also reflected in case of cost benefit (C:B) ratio wherein IPM farmers registered higher C:B ratio, whereas non-IPM farmers had relatively lower C:B ratio. Adoption of IPM technology resulted in reducing the number of sprays to 5-6 from average number of 15 (Table 2) in FP fields. Halder et al. (2018) and Sardana et al. (2017) also reported higher yields in bitter gourd and in onion seed crop fields of IPM than non-IPM fields, respectively. Feedback from IPM farmers indicated the increased knowledge, awareness and adoption of 80% of the IPM components for bitter gourd by majority of the adopted farmers. Adoption of IPM technology enabled the farmers also to differentiate between the pests and bioagents and avoidance of widely prevalent practice of using mixtures of pesticides.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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EFFICACY OF TAKUMI 20% WG AGAINST RICE STEM BORERS AND LEAF FOLDER

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ABSTRACT

Insect pests pose major menace to the rice production and amongst these stem borer *Scirpophaga incertulas* (Walker) and rice leaf folder *Cnaphalocrocis medinalis* (Guen.) are important and cause enormous yield losses. This study evaluates the efficacy of newer insecticide, Takumi 20WG (flubendiamide 20%) against these, compared with checks, Fame 480SC (flubendiamide 39.35%) and Coragen 18.5SC (chlorantraniliprole 18.5%). The insecticides were applied on economic threshold basis (ETL) for stem borers (2% deadhearts on basmati and 5% deadhearts on non-basmati) and/or leaf folders (10% damaged leaves) in randomized block design. The results revealed that Takumi 20WG @ 125 g/ ha was statistically at par with the checks, Fame 480SC and Coragen 18.5SC but significantly superior to its lower dosages of 100 and 75 g/ ha.

Key words: Rice, basmati, Takumi 20WG, flubendiamide 20%, Fame 480SC, flubendiamide 39.35%, Coragen 18.5S, chlorantraniliprole *Scirpophaga incertulas*, *Cnaphalocrocis medinalis*, deadhearts, white ears, yield

Rice (*Oryza sativa* L.) is one of the most important cereal (FAO, 2004) that accounts for >50% of the daily calorie intake (Khush, 2005), and it is a chief staple food crop (Mathur et al., 1999; Sarwar, 2012). In India, it is cultivated almost in one-fourth of the total cropped area providing food to about half of the Indian population (Seni and Naik, 2018). Approximately 300 species of insect pests attack rice and amongst them only 23 species cause notable damage (Pasalu and Katti, 2006). Among these, stem borer, *Scirpophaga incertulas* (Walker) is the most important and devastating insect pest causing yield losses up to 87.66% (Pallavi et al., 2017). When the infestation occurs at the flowering stage, the ear heads become chaffy, whitish, erect called, "white ears" (Jadhao and Khurad, 2012; Muralidharan and Pasalu, 2006). Another important insect causing yield losses is the rice leaf folder *Cnaphalocrocis medinalis*, which is widespread with high yielding rice varieties and accompanying changes in the cultural practices (Teng et al., 1993). Insecticides are always used against such pests and indiscriminate use of these has resulted in the failure of control due to development of resistance to insecticides. Under such conditions, use of novel insecticides having specificity towards target insects is the need (Narayanaswamy, 1995). Hence, the present study to evaluate the comparative efficacy of some newer insecticide against stem borer and leaf folder in rice and basmati.

MATERIALS AND METHODS

The experiment was conducted during the main cropping season of 2019/2020. Takumi 20WG (flubendiamide), a phthalic acid diamide group of insecticide (green toxicity category) of M/s Rallis India Ltd., Bangalore was evaluated @ 75, 100 and 125 ml/ ha (15, 20 and 25 g a.i. ha^{-1}) against stemborers and leaf folder infesting rice/basmati in four research trials (02 trials, each on basmati and non-basmati) conducted at Ludhiana, Patiala and Rupnagar, Punjab during *Kharif* 2019 and 2020 crop seasons and compared with the checks, Fame 480SC (flubendiamide 39.35%) @ 50 ml/ha (24 g a.i. ha^{-1}) and Coragen 18.5SC (chlorantraniliprole 18.5%) @ 150 ml/ ha (30 g a.i. ha^{-1}). An untreated control was also kept for comparison. Flubendiamide 20WG is a registered insecticide by Central Insecticide Board and Registration Committee at a dose of 25g a.i./ ha against rice leaf folder and stem borer in rice. The variety for non-basmati was PR 121 and Pusa Basmati 1121 for basmati. The experiments were laid out in randomized block design with three replications and insecticides were applied on economic threshold basis (ETL) for stemborers (2% deadhearts on *basmati* and 5% deadhearts on non-basmati) and leaf folders (10% damaged leaves). All other agronomic practices were done as recommended for the crop by Punjab Agricultural University, Ludhiana, Punjab. The observations on stem borer incidence as dead-hearts at vegetative stage and leaf folder damage as damaged leaves were recorded one day before insecticide application and then 5 and 10 days after the application. White-ears incidence (caused by stemborers) was recorded about a week before the crop harvest. Natural

Table 1. Efficacy of flubendiamide against rice stem borers and leaf folder and yield (q/ha) in Punjab- kharif 2019, 2020

Treatment	Dose		2019		2020		Pooled Mean	PROC
	g a.i./ha	per ha	Expt. 1	Expt. 2	Expt. 3	Expt. 4		
			Deadhearts (%) Before Insecticide Application					
Takumi 20WG (flubendiamide 20%)	15	75 g	3.16± 0.00[a]	4.36± 0.01[a]	6.02± 0.01[a]	2.82± 0.00[a]	4.09± 0.01[a]	--
	20	100 g	3.18± 0.01[a]	4.35± 0.00[a]	6.01± 0.01[a]	2.79± 0.00[a]	4.08± 0.02[a]	--
	25	125 g	3.27± 0.00[a]	4.49± 0.00[a]	6.00± 0.02[a]	2.81± 0.00[a]	4.14± 0.01[a]	--
Fame 480SC (flubendiamide 39.35%)	24	50 ml	3.26± 0.02[a]	4.41± 0.01[a]	5.96± 0.00[a]	2.84± 0.01[a]	4.12± 0.01[a]	--
Coragen 18.5SC (chlorantraniliprole)	30	150 ml	3.20± 0.01[a]	4.45± 0.02[a]	5.97± 0.00[a]	2.90± 0.02[a]	4.13± 0.01[a]	
Untreated Control	--	---	3.25± 0.00[a]	4.67± 0.01[a]	6.01± 0.01[a]	2.91± 0.01[a]	4.21± 0.01[a]	--
			Deadhearts (%) 5 DAA					
Takumi 20WG (flubendiamide 20%)	15	75 g	2.93± 0.09[c]	4.10± 0.13[b]	4.32± 0.08[b]	2.54± 0.29[c]	3.47± 0.19[c]	30.18
	20	100 g	2.17± 0.14[b]	2.60± 0.32[a]	4.15± 0.17[b]	1.77± 0.25[b]	2.68± 0.30[b]	46.08
	25	125 g	1.36± 0.09[a]	2.07± 0.18[a]	1.80± 0.21[a]	1.17± 0.10[a]	1.60± 0.22[a]	67.81
Fame 480SC (flubendiamide 39.35%)	24	50 ml	1.34± 0.11[a]	2.11± 0.09[a]	1.78± 0.15[a]	1.20± 0.05[a]	1.61± 0.10[a]	67.61
Coragen 18.5SC (chlorantraniliprole)	30	150 ml	1.36± 0.09[a]	1.98± 0.18[a]	1.76± 0.17[a]	1.18± 0.10[a]	1.57± 0.21[a]	68.41
Untreated Control	--	---	4.05± 0.14[d]	5.49± 0.31[c]	7.12± 0.07[c]	3.24± 0.15[d]	4.97± 0.19[d]	--
			Deadhearts (%) 10 DAA					
Takumi 20WG (flubendiamide 20%)	15	75 g	3.04± 0.10[c]	3.83± 0.13[b]	4.47± 0.13[b]	2.51± 0.03[c]	3.46± 0.14[c]	42.24
	20	100 g	1.36± 0.11[b]	1.52± 0.18[a]	4.19± 0.13[b]	1.20± 0.10[b]	2.07± 0.20[b]	65.44
	25	125 g	0.76± 0.00[a]	0.99± 0.05[a]	1.48± 0.10[a]	0.61± 0.08[a]	0.88± 0.05[a]	85.31
Fame 480SC (flubendiamide 39.35%)	24	50 ml	0.78± 0.10[a]	0.95± 0.04[a]	1.16± 0.11[a]	0.59± 0.10[a]	0.87± 0.13[a]	85.48
Coragen 18.5SC (chlorantraniliprole)	30	150 ml	0.81± 0.12[ab]	1.01± 0.09[a]	1.13± 0.17[a]	0.64± 0.05[a]	0.90± 0.10[a]	84.98
Untreated Control	--	---	4.98± 0.13[d]	5.64± 0.15[c]	8.04± 0.21[c]	4.30± 0.14[d]	5.99± 0.11[d]	--
			White ears (%) At Harvest					
Takumi 20WG (flubendiamide 20%)	15	75 g	4.31± 0.15[b]	5.05± 0.17[b]	6.20± 0.10[b]	4.98± [c]	5.14± 0.12[c]	30.35
	20	100 g	2.73± 0.11[a]	3.18± 0.13[a]	5.76± 0.15[b]	3.93± 0.10[b]	3.90± 0.09[b]	47.15
	25	125 g	2.56± 0.07[a]	3.03± 0.10[a]	2.58± 0.12[a]	3.06± 0.11[a]	2.81± 0.10[a]	61.92
Fame 480SC (flubendiamide 39.35%)	24	50 ml	2.52± 0.12[a]	3.08± 0.12[a]	2.54± 0.15[a]	4.00± 0.19[a]	2.81± 0.11[a]	61.92
Coragen 18.5SC (chlorantraniliprole)	30	150 ml	2.65± 0.10[a]	3.04± 0.05[a]	2.51± 0.10[a]	3.20± 0.09[ab]	2.85± 0.10[a]	61.38
Untreated Control	--	---	7.25± 0.20[c]	7.67± 0.31[c]	7.58± 0.25[c]	7.03± 0.21[d]	7.38± 0.19[d]	--
			LF Damaged leaves (%) Before Insecticide Application					
Takumi 20WG (flubendiamide 20%)	15	75 g	9.02± 0.00[a]	11.13± 0.00[a]	8.02± 0.02[a]	11.22± 0.02[a]	9.85± 0.02[a]	--
	20	100 g	9.06± 0.01[a]	11.27± 0.00[a]	8.00± 0.03[a]	11.43± 0.04[a]	9.94± 0.03[a]	--
	25	125 g	8.90± 0.02[a]	11.14± 0.02[a]	8.01± 0.01[a]	11.35± 0.03[a]	9.85± 0.05[a]	--
Fame 480SC (flubendiamide 39.35%)	24	50 ml	9.14± 0.05[a]	11.20± 0.03[a]	8.04± 0.00[a]	11.51± 0.02[a]	9.97± 0.02[a]	--
Coragen 18.5SC (chlorantraniliprole)	30	150 ml	9.15± 0.04[a]	11.38± 0.03[a]	8.03± 0.02[a]	11.55± 0.04[a]	10.03± 0.02[a]	
Untreated Control	--	---	9.08± 0.01[a]	11.44± 0.00[a]	7.97± 0.03[a]	11.60± 0.03[a]	10.02± 0.01[a]	--
			Leaffolder damaged leaves (%) 5 DAA					
Takumi 20WG (flubendiamide 20%)	15	75 g	6.35± 0.11[c]	7.62± 0.14[c]	4.88± 0.19[b]	8.06± 0.14[c]	6.73± 0.12[c]	41.48
	20	100 g	2.87± 0.11[b]	3.92± 0.13[b]	4.48± 0.21[b]	3.84± 0.21[b]	3.78± 0.18[b]	67.13
	25	125 g	1.95± 0.09[a]	2.87± 0.15[a]	2.12± 0.09[a]	2.92± 0.12[a]	2.47± 0.15[a]	78.52
Fame 480SC (flubendiamide 39.35%)	24	50 ml	1.96± 0.10[a]	2.92± 0.14[a]	2.11± 0.10[a]	2.89± 0.14[a]	2.47± 0.19[a]	78.52
Coragen 18.5SC (chlorantraniliprole)	30	150 ml	1.86± 0.11[a]	2.73± 0.09[a]	2.10± 0.10[a]	2.86± 0.15[a]	2.38± 0.11[a]	79.30
Untreated Control	--	---	10.27± 0.17[d]	13.11± 0.20[d]	9.27± 0.14[c]	13.34± 0.16[d]	11.50± 0.17[d]	--
			Leaffolder damaged leaves (%) 10 DAA					
Takumi 20WG (flubendiamide 20%)	15	75 g	6.58± 0.11[c]	8.97± 0.14[c]	5.34± 0.10[b]	8.15± 0.14[c]	7.26± 0.17[c]	47.69
	20	100 g	2.54± 0.10[b]	3.89± 0.13[b]	5.14± 0.14[b]	3.72± 0.17[b]	3.82± 0.16[b]	72.48
	25	125 g	1.38± 0.09[a]	2.18± 0.11[a]	1.26± 0.15[a]	1.97± 0.14[a]	1.70± 0.09[a]	87.75
Fame 480SC (flubendiamide 39.35%)	24	50 ml	1.48± 0.05[a]	2.17± 0.13[a]	1.23± 0.10[a]	2.04± 0.13[a]	1.73± 0.08[a]	87.54

(contd.)

Coragen 18.5SC (chlorantraniliprole)	30	150 ml	1.52± 0.09[a]	2.21± 0.15[a]	1.21± 0.09[a]	2.13± 0.11[a]	1.77± 0.13[a]	87.25
Untreated Control	--	---	12.80± 0.19[d]	15.18± 0.22[d]	11.52± 0.17[c]	16.01± 0.15[d]	13.88± 0.13[d]	--
Natural Enemies (Spiders/ hill) at 10 DAA								
Takumi 20WG (flubendiamide 20%)	15	75 g	0.53± 0.05[a]	0.57± 0.00[a]	0.67± 0.01[a]	0.53± 0.04[a]	0.58± 0.03[a]	--
	20	100 g	0.57± 0.01[a]	0.53± 0.02[a]	0.65± 0.04[a]	0.57± 0.02[a]	0.58± 0.03[a]	--
	25	125 g	0.53± 0.03[a]	0.53± 0.02[a]	0.65± 0.04[a]	0.53± 0.03[a]	0.56± 0.00[a]	--
Fame 480SC (flubendiamide 39.35%)	24	50 ml	0.50± 0.01[a]	0.53± 0.00[a]	0.65± 0.02[a]	0.53± 0.04[a]	0.55± 0.03[a]	--
Coragen 18.5SC (chlorantraniliprole)	30	150 ml	0.50± 0.04[a]	0.53± 0.02[a]	0.67± 0.01[a]	0.57± 0.02[a]	0.57± 0.03[a]	--
Untreated Control	--	---	0.57± 0.00[a]	0.57± 0.02[a]	0.69± 0.03[a]	0.57± 0.00[a]	0.60± 0.00[a]	--

			Yield							
			Mean Yield* (q/ha)				Pooled Yield (q/ha)		% Increase over control	
			Expt. 1	Expt. 2	Expt. 3	Expt. 4	Non-Basmati	Basmati	Non-Basmati	Basmati
Takumi 20WG (flubendiamide 20%)	15	75 g	30.33± 0.12[a]	71.07± 0.19[c]	53.20± 0.10[b]	29.83± 0.12[b]	62.13± 0.09[c]	30.08± 0.20[c]	+ 5.4	+ 17.7
	20	100 g	33.33± 0.09[a]	74.93± 0.23[b]	55.00± 0.20[b]	32.00± 0.15[a]	64.97± 0.12[b]	32.67± 0.15[ab]	+ 10.3	+ 17.0
	25	125 g	34.50± 0.16[a]	78.40± 0.18[a]	62.20± 0.19[a]	34.17± 0.15[a]	70.30± 0.15[a]	34.33± 0.11[a]	+ 19.3	+ 22.9
Fame 480SC (flubendiamide 39.35%)	24	50 ml	34.67± 0.10[a]	78.13± 0.15[a]	62.33± 0.11[a]	33.83± 0.09[a]	70.23± 0.19[a]	34.25± 0.15[ab]	+ 19.2	+ 22.7
Coragen 18.5SC (chlorantraniliprole)	30	150 ml	34.17± 0.12[a]	77.87± 0.10[ab]	62.57± 0.19[a]	33.33± 0.15[a]	70.22± a	33.75± 0.13[ab]	+ 19.2	+ 20.9
Untreated Control	--	---	28.33[c]	68.93[c]	48.93[c]	27.50[c]	58.93[d]	27.92[d]	--	--

*Means within same column followed by same letter not significantly different (Duncan's MRT, $p < 0.05$); PROC: % reduction over control

enemies were also recorded ten days after insecticide application. The yield (q/ ha) was also recorded at harvest. Pesticide residue studies at proposed and double the proposed dose were also conducted at PAU, Ludhiana. Data were subjected to ANOVA with statistical software SPSS v 20.0 (SPSS, 2011). The comparison of means was done using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The plots treated with Takumi 20WG @ 125 g/ ha recorded 1.36 & 2.07 % deadhearts in trials conducted at Ludhiana and Patiala during 2019, and 1.80 & 1.17 % at Ludhiana and Ropar during 2020, after 5 days of insecticide application, with an overall pooled mean of 1.60% which were statistically at par with the checks, Fame 480SC and Coragen 18.5SC (Table 1). Arulkumar et al. (2019) evaluated the foliar spray of flubendiamide 20WG. The present findings also corroborate with results of Sahu et al., (2020) on chlorantraniliprole either as granule or as spray against rice stemborer. The observations recorded in the present investigation after 10 days of insecticide application, reported overall 0.88% mean deadhearts with Takumi 20WG @ 125 g/ ha which was statistically at par with Fame 480SC and Coragen 18.5SC. Present results agree with those of Shyamrao and Raghuraman (2019) that flubendiamide 20WG @ 25 g a.i./ ha is the most effective against stem borer, followed by chlorantraniliprole 18.5SC @ 30 g a.i./ha (4.23%). Similar results were obtained by Devi and Singh (2016) with flubendiamide 39.35SC @ 24 g a.i./ha. Chormule et al. (2014) observed that Takumi 20WG proved to be the most effective in reducing the stem borer. Takumi 20WG @ 125 g/ha with an overall mean of 2.81 % white ears, was statistically at par with Fame 480SC and Coragen 18.5SC (Table 1). Suri (2011) and Suri and Brar (2012) compared chlorantraniliprole at different doses with thiocyclam hydrogen oxalate @ 400 g a.i.ha^{-1} and cartap hydrochloride @ 1000 g a.i.ha^{-1} and reported that chlorantraniliprole is significantly superior in reducing deadhearts and white ear heads effectively.

Against leaf folder, Takumi 20WG @ 125 g/ ha recorded 1.95 & 2.87% damage in trials conducted at Ludhiana and Patiala during 2019 and 2.12 & 2.92% damage at Ludhiana and Ropar during 2020, after 5 days of insecticide application, with an overall pooled mean of 2.47% which were statistically at par with Fame 480SC and Coragen 18.5SC (Table 1). Zala and Sipai (2021) reported that application of flubendiamide 20WG @ 25 g a.i. ha^{-1} recorded 12.51% leaf damage due to *C. medinalis*. Jaglan and Chaudhary (2021) reported that foliar spray of flubendiamide 20WG @

25 g a.i. ha^{-1} effectively reduced the infestation of leaf folder and yellow stem borer. Takumi 20WG @ 125 g/ ha (25 g a.i. ha^{-1}) gave yields at par with Fame 480SC and Coragen 18.5SC at all the test locations; pooled data revealed that an yield of 70.30 q/ ha was obtained in case of non-basmati rice with Takumi 20WG @ 125 g/ ha. Similar was the case with Basmati rice, yield of 34.33 q/ ha was observed with Takumi 20WG @ 125 g/ ha, that was on par with Fame 480 SC (34.25 q/ ha) and Coragen 18.5SC (33.75 q/ ha). These results are in agreement with those of Shyamrao and Raghuraman (2019) using flubendiamide 20WG @ 25 g ai./ ha. Ghoghari et al. (2019) observed for control of stem borer in rice, flubendiamide 20 WG gave maximum yield. Sridhar and Sharma (2015) also observed that flubendiamide 20WG @ 60 g a.i. ha^{-1} was the most effective. In non-basmati rice also, similar yields were observed with Takumi 20WG. The spider counts recorded in various treatments over the test locations ranged from 0.50 to 0.69/ hill at 10 days after insecticide application (Table 1). These results agree with those of Sekh et al., (2007) that flubendiamide 480SC @ 24 and 30 g a.i./ ha was soft to egg parasitoids of yellow stem borer. Similar results were also reported by Tohnishi et al. (2005) Kubendran et al. (2006) and Thilagam et al. (2006) with flubendiamide being the least toxic against beneficial arthropods.

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PHYTOTOXICITY EFFECT OF PONGAMIA OIL ON CHILLI

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ABSTRACT

This study evaluated the phytotoxicity potential of pongamia oil on chilli plants. The pongamia oil at 0.5, 1.0, 2.5, 5.0 and 10% concentrations were evaluated under field conditions. The dose @ 0.5, 1.0 and 2.5% were observed to be safe with no phytotoxicity symptoms. Pongamia oil @ 5.0 and 10% caused the moderate and high phytotoxicity interfering with photosynthesis and plant growth.

Key words: *Capsicum annuum*, sucking pests, pongamia oil, essential oil, karanjin, phytotoxicity, photosynthesis, plant growth, yield, benefit cost ratio

India is the world's largest producer, consumer and exporter of chilli (*Capsicum annuum* L.) . It is an essential ingredient of Indian curry. Major losses in the chilli yield have been recorded due to thrips (*Scirtothrips dorsalis* Hood), mites (*Polyphagotarsonemus latus* Banks) and aphids (*Myzus persicae* Sulzer, *Aphis gossypii* Glover). Repeated application of insecticides for their management leads to several ill effects. To avoid the harmful effects of synthetic insecticides considerable effort has been devoted to using ecofriendly products from natural sources. Botanicals are now emerging as the prime means to protect crops (Kovarikova and Pavela, 2019). Essential oils from aromatic plants serve as source of biopesticides (Abd-ElGawad et al., 2021). Pongamia oil, rich in karanjin has excellent biological activity, and is a good synergist and has antifeedant, oviposition deterrent, ovicidal and insecticidal properties (Kumar et al., 2006). A combination of pongamia oil + neem oil + cotton seed oil + citronella oil (50:25:15:10 ratio) @ 2.0% concentration has been found effective against sucking pests in the chilli ecosystem (Gadge et al., 2021). Though pongamia oil offers good insecticidal potential along with several advantages over conventional pesticides, it is observed to have phytotoxicity effects. This study evaluates the phytotoxicity of pongamia oil on chilli under field conditions.

MATERIALS AND METHODS

Field experiment was laid out at the College of Horticulture, Bagalkote, Karnataka (16° 9' 52" N, 75° 36' 51" E, 542 masl) during kharif 2019-20. Randomized block design (RBD) with seven treatments and three replications, with a plot size of 2.4 x 2.1 m leaving a gangway of one meter around the plots was followed. Thirty days old chilli seedlings of the variety Sitara Gold (Monsanto) were transplanted at a spacing of 60 x 30 cm, and the crop raised following recommended package of practices of UHS, Bagalkote (Anonymous, 2018). The treatments were imposed using a knapsack hydraulic sprayer at a spray volume of 500 l/ ha. The first spray was given ten days after transplanting (DAT) and subsequent ones at an interval of ten days. Pongamia oil was procured from the market, and used at five concentrations of 0.5, 1.0, 2.5, 5.0 and 10% mixed with a 0.3% spreader concentration. Phytotoxicity effect with observations from ten randomly tagged plants leaving the border rows, were recorded a day before, 3, 7 and 10 days after each spray. Symptoms like leaf margin necrosis (browning) or chlorosis (yellowing), brown or yellow leaf spots or patches, leaf cupping or twisting and plant stunting or plant death were scored in a 0 - 10 scale (Ambarish et al., 2017). Subsequently, green chillies were harvested separately at each picking and yield was recorded as kg/ plot and computed on ha basis. The B: C ratio was worked out, and the data were subjected to ANOVA with treatment means compared by Duncan's Multiple Range Test (DMRT, p=0.05).

RESULTS AND DISCUSSION

A day before spraying all the plots were observed with

Table 1. Evaluation of pongamia oil for its phytotoxicity effect on chilli

Tr. No.	Treatment	Dosage	Phytotoxicity symptoms (0-10 scale)																				Green chilli yield (t/ ha)	BCR
			Chlorosis/ yellowing					Leaf cupping/ twisting					Leaf dropping					Leaf necrosis						
			1 DAS	3 DAS	7 DAS	10 DAS	Mean	1 DAS	3 DAS	7 DAS	10 DAS	Mean	1 DAS	3 DAS	7 DAS	10 DAS	Mean	1 DAS	3 DAS	7 DAS	10 DAS	Mean		
T_1:	Pongamia oil	0.5%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.50 (3.00)^{ab}	1.53
T_2:	Pongamia oil	1.0%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.63 (3.01)^{ab}	1.57
T_3:	Pongamia oil	2.5%	0	1	1	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.17 (3.11)^{a}	1.63
T_4:	Pongamia oil	5.0%	1	3	3	4	2.75	1	3	3	4	2.75	2	2	3	4	2.75	0	1	2	2	1.25	6.05 (2.56)^{bc}	1.05
T_5:	Pongamia oil	10%	1	3	5	7	4	6	8	9	10	8.25	4	8	9	10	7.75	8	9	10	10	9.25	5.36 (2.42)^{c}	0.88
T_6:	NSPE	5%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.02 (3.21)^{a}	1.82
T_7:	Untreated control	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.14 (2.76)^{abc}	1.29
SEm±			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	-
CD at 5%			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.47	-
CV(%)			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.30	-

(Mean of all 5 sprays); Phytotoxicity visual ratings based on a grade of 0-10, in which 0 is no phytotoxicity (Ambarish et al., 2017); Each value mean of 3 replications. NSPE-Neem Seed Powder Extract; DAS-Days After Spraying; BCR- Benefit Cost Ratio

normal plants showing no phytotoxicity symptoms, and with post treatment too pongamia oil @ 0.5 and 1.0% did not exhibit phytotoxicity symptoms. Plants treated with 2.5% pongamia oil showed low yellowing or chlorosis after 3 and 7 days of the first spray (DAS) (phytotoxicity score 1), which recovered to normal after 10 DAS; pongamia oil @ 5% recorded a phytotoxicity score of 2.75 with chlorosis or yellowing, leaf cupping or twisting, and leaf dropping; at 10%, score was 4 for chlorosis or yellowing, 8.25 for leaf cupping or twisting, 7.75 for leaf dropping and 9.25 for leaf necrosis. Thus, pongamia oil @ 5 and 10% is highly phytotoxic, and at <2.5%, it is safe. These results agree with those of Rolli et al. (2014) who revealed that higher contents of monoterpene alcohols, aldehydes and phenylpropanoids in an essential oil may be an indicator of phytotoxicity. Poonpaiboonpipat et al. (2013) observed decrease in chlorophyll a, b and carotenoid contents indicating that essential oil interfered with photosynthesis. Kumar et al. (2019) observed that recommended doses of castor, pongamia, sesame and neem oil and its formulated products did not cause any problem on tender cotton leaves. Galhiane et al. (2012) showed that castor oil is non-phytotoxic at concentrations that are effective against mites and aphids. When the temperature is high throughout the day, the oil disappears more rapidly and phytotoxic symptoms are reduced in plants. Loss of oil from treated plant surfaces is more rapid in tropical and subtropical than in temperate regions (Beattie, 2005). The phytotoxic potential of essential oil depends not only on the dose, but also on the plant species (Synowiec et al., 2016).

A significant difference in the yield of green chillies was observed with pongamia oil treatment; maximum yield of 10.02 t/ ha was obtained from NSPE treated plot which was statistically on par with pongamia oil @ 2.5% with 9.17 t/ ha, along with no phytotoxicity @ 2.5% concentration. These results corroborate with those of Gadge et al. (2021) that pongamia oil + neem oil + cotton seed oil + citronella oil (50:25:15:10 ratio) and pongamia oil + neem oil (50:50 ratio) @ 2.0% yielded significantly higher yield of chilli. The yield was however low from the plots treated with pongamia oil @ 5.0 and 10%, which is due to their phytotoxicity. Meena and Tayade (2017) obtained maximum yield and BC ratio with pongamia oil @ 4.0%. Zeeshan and Kudada (2019) obtained more chilli yield with neem oil @ 0.03 and karanj oil @ 0.15%. Thus, pongamia oil @ 0.5, 1.0 and 2.5% is safe for chilli plants.

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POPULATION DYNAMICS OF ONION THRIPS *THRIPS TABACI* LINDEMAN

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ABSTRACT

A field experiment was conducted at Entomology farm, Faculty of Agriculture, SKUAST-K during rabi, 2019-20 to study the population dynamics of *Thrips tabaci* Lindeman in onion. The incidence of adults (31.00/ plant) and immatures (61.00/ plant) was observed from 9th standard meteorological week (SMW). It reached a peak on 22nd and 21st SMW and declined thereafter. The incidence revealed a positive correlation with temperature, rainfall and evening RH, and a negative one with morning RH; however, with nymphs it was a positive one with temperature and evening RH, and a negative one with rainfall and morning RH.

Key words: *Allium cepa*, *Thrips tabaci*, seasonal incidence, correlation, weather parameters, temperature, relative humidity, rainfall

Onion (*Allium cepa* L.) is an important horticultural crop grown in 3.10 thousand ha (Anonymous, 2018), but its productivity is low in India, with 10-15% loss due to insect and non-insect pests and weather factors (Muthuram et al., 2017). Among the insect pests onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) is the major one with the affected plant exhibiting curling and drying of leaves which also affects the size and weight of the bulb. The bulb formation stage is most susceptible to thrips damage and as a result, the size and weight of onion bulb gets reduced. The yield losses in onion as a result of thrips damage can vary from 34.5 to 43% (Fournier et al., 1995) and can be up to 90% (Gupta et al., 1984). Therefore, the present study on its population dynamics at Wadura in Jammu and Kashmir.

MATERIALS AND METHODS

Field experiment was conducted at the Division of Entomology, Faculty of Agriculture, Wadura campus, Sopore, during rabi 2019-20. The seeds of variety "Yellow globe" were sown in mid-October under greenhouse conditions, with the seedlings transplanted during November, 2019 as per the package of practices recommended by SKUAST-K. The plot size was 12 x 4 m^2 with 16 rows, with row to row and plant to plant distance maintained at 15x 30 cm, respectively. Observation on thrips incidence (adult and immature stages) was recorded soon after the appearance of the thrips and continued till the harvest. The counts were made by randomly selecting three plants from each sampling unit and number of thrips counted with a magnifying lens (10x). The means computed from the data obtained at weekly intervals from 14DAT (days after transplanting) up to harvesting were used for analysis. The weather data was obtained from the meteorological observatory located at Faculty of Horticulture, SKUAST-K. The data on incidence were correlated with temperature, relative humidity and rainfall, and correlation (r) and regression coefficients (R^2) were computed.

RESULTS AND DISCUSSION

Table 1 reveals that the adults and immatures of *T. tabaci* appeared from 9th standard meteorological week (SMW) @ 0.33± 0.57 adults and 1.67± 0.57 nymphs/ plant; thereafter, incidence increased and reached its peak in 22nd SMW (31.00± 1.00- adults) and 21st SMW (61.00± 3.60- nymphs); and declined to 27.33± 0.57 adults and 40.00± 1.00 nymphs at 24th SMW. These observations corroborate with those of Khan et al. (2015) in the Khyber Pakhtunkhwa Province. El-Sherif and Mahmoud (2008) observed a declining trend by the end of May (21st SMW) similar to those of Reuda and Shelton (2003). Kannan and Mohamed (2001) noticed a gradual increase from March onwards, while Ullah et al. (2010) observed its activity on 3rd February (5th SMW) with a peak during last week of April (17th SMW). The correlation of incidence with weather factors revealed that adults of *T. tabaci* had a positive correlation with rainfall (r=0.033), evening RH (r=0.062), maximum temperature (r=0.805) and minimum temperature (r=0.966); and negative one with morning RH (r=-0.518). However, the nymphs revealed a positive

Table 1. Incidence of *Thrips tabaci* on onion crop under field conditions during rabi, 2019-20

SMW	Mean no. of adults/ plant*	Mean no. of nymphs/ plant*	Mean (adults+ nymphs)
09	0.33± 0.33	1.67± 0.57	2.00± 0.66
10	1.67± 0.33	4.00± 1.00	5.67± 1.16
11	4.00± 0.57	7.00± 1.00	11.00± 1.50
12	7.00± 0.57	12.67± 2.08	19.67± 2.83
13	8.33± 0.66	16.33± 2.08	24.67± 4.00
14	13.00± 1.15	20.67± 2.51	33.67± 3.83
15	14.00± 0.57	25.67± 3.51	39.67± 5.83
16	16.67± 0.66	29.67± 2.51	46.33± 6.5
17	19.00± 0.57	32.33± 2.08	51.33± 6.66
18	22.00± 0.57	36.67± 2.08	58.66± 7.33
19	24.33± 0.88	41.00± 1.00	65.33± 8.33
20	27.00± 0.57	46.67± 1.52	73.66± 9.83
21	29.00± 0.57	61.00± 3.60	90.00± 16.00
22	31.00± 0.57	59.67± 1.52	90.67± 14.33
23	29.00± 0.57	47.00± 2.00	76.00± 9.00
24	27.33± 0.33	40.00± 1.00	67.33± 6.33
Mean± S.E.	17.10± 0.59	30.13± 1.08	47.22± 6.51

Correlation coefficients (r)

Parameters	Adults	Immatures
Rainfall (mm)	0.033 (0.901)	-0.004 (0.988)
Maximum temperature (°C)	0.805* ($1.70e^{-04}$)	0.791*($2.63e^{-04}$)
Minimum temperature (°C)	0.966* ($1.41e^{-09}$)	0.920* ($4.47e^{-07}$)
Morning RH (%)	-0.518* (0.039)	-0.540* (0.030)
Evening RH (%)	0.062 ($8.21e^{-01}$)	0.047 ($8.63e^{-01}$)

Regression equation/ coefficient of multiple determination (R^2)

	Linear regression equation	Coefficient (r)	Coefficient of determination (R^2)	Coefficient of variance (%)
Thrips (Adults)	$Y_1=100.69-272X_1-887X_2+3.29X_3-1.02X_4+0.72X_5$	0.979	0.959	95%
Thrips (Immatures)	$Y_2=196.61-392X_1+0.07X_2+3.18X_3-2.77X_4+0.88X_5$	0.950	0.903	90%

*Mean of 3 replications± S.E; SMW: Standard Meteorological week; *Significant at $p \leq 0.05$; RH= Relative humidity where, Y=Mean number of thrips population/ leaf; X_1= Rainfall (mm); X_2=Maximum temperature (°C); X_3=Minimum temperature (°C); X_4=RH morning (%); X_5=RH evening (%).

correlation with evening RH (r=0.047), maximum temperature (r=0.791) and minimum temperature (r=0.920), and negative one with rainfall (r=-0.004) and morning RH (r=-0.540). These observations derive support from Moraiet and Ansari (2014) on the positive correlation with temperature and negative correlation with RH; Lorine et al. (1986) observed that incidence decreases with rainfall and increases with temperature; Vinuthan et al. (2018) observed a negative correlation of RH and rainfall, and a significant positive correlation with temperature; and temperature was found positively correlated in rabi, and in kharif season, there was a significant negative correlation with rainfall (Sudhir 2011). Low temperature could arrest development of thrips (Stacey and Fellowes, 2002). Murai (2000) suggested that that the intrinsic rate of growth of *T. tabaci* at the temperatures lower than 25°C is due to the advantage of the thelytokous reproduction in western Japan, and the opposite result at 30°C is due to hatchability of *T. tabaci*. The data on linear regression equations (Y=a+bx) for adults and immatures of *T. tabaci* during 2019-20 was found to

be Y_1=100.69-272X_1-887X_2+3.29X_3-1.02X_4+0.72X_5 and Y_2=196.61-392X_1+0.07$_x$2+3.18X_3-2.77X_4+0.88X_5, respectively. The corresponding correlation coefficient (R^2) values for adult and immature thrips were worked out to be 0.959 and 0.903, respectively. The overall impact of weather on adult thrips was 95%, while it was 90% with immatures.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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SEASONAL INCIDENCE OF PEA LEAF MINER *CHROMATOMYIA HORTICOLA* GOUREAU INFESTING GARDEN PEA

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ABSTRACT

The seasonal incidence of pea leaf miner *Chromatomyia horticola* Goureau, on garden pea (*Pisum sativum* L.) was assessed during 2017 and 2018 at the research farm of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha. The pooled data revealed that the incidence show continuous fluctuation, with first appearance being during the 49th standard meteorological week (SMW)- 0.95 miners/ leaf which reached its peak (11.00 miners/ leaf) during the 4th SMW, then reached to minimum (1.26 miners/ leaf) during the 12th SMW. The correlation studies indicated highly significant negative association between incidence and maximum temperature (-0.714), significant positive one with evening relative humidity (RH) (0.544*); and non-significant ones with minimum temperature (-0.385) and wind speed (-0.74), morning RH (0.258), sunshine (0.013) and rainfall (0.356).**

Key words: *Chromatomyia horticola*, *Pisum sativum*, incidence, population dynamics, correlation coefficients, regression, weather parameters, temperature, humidity

Garden pea (*Pisum sativum* L.) is an important rabi grain legume crop, and in Jammu and Kashmir, it is grown over an area of 2.79 thousand ha, with an annual production of 58.08 thousand mt (Anonymous, 2017). The leaf miner *Chromatomyia horticola,* Goureau (Diptera: Agromyzidae) is an important ipest of this crop. Leaf miners are herbivorous insects that pass their larval stages inside the leaf (Anderson et al., 2002). In case of severe attack entire leaf is filled with the mines and 86-93% leaves are affected (Atwal et al., 1969). The destruction of the chlorophyll containing tissue affects the growth and yield (Vishwanath and Agrawal, 1982). Rai and Ram (1997) observed the weather parameters as the major regulatory factors for pea leaf miner infestation. The present study analyses its seasonal incidence and evaluates its population dynamics.

MATERIALS AND METHODS

A field experiment was conducted during 2017 to 2018 on *P. sativum* at the research farm of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu Chatha. The variety Arkel was raised in a plot size of 10x 3 m in a completely randomized block design with three replications. All the recommended agronomic practices were followed except plant protection. Weekly observations were recorded on 10 randomly selected plants. The incidence/ plant was worked out by counting the number of larvae and pupae on randomly selected 5 leaves/ plant. Weather data on mean temperature (maximum/ minimum-°C), mean relative humidity- RH (morning and evening), and rainfall (mm) were obtained from the Agrometeorological Section, Division of Agronomy, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu. The analysis of data was done using SPSS software package.

RESULTS AND DISCUSSION

The pooled data revealed that the first incidence of *C. horticola* was during the 49th standard meteorological week (SMW) at 0.95 leaf miners/ plant (Fig. 1); it increased gradually and reached to a peak (11.00 leaf miners/ plant) during the 4th SMW, and then decreased to a minimum of 1.26 leaf miners/ plant during the 12th SMW. Peak activity was during first week of October and January i.e. 40th and 1st SMW with no infestation during April and May (Hemalatha and Maheswari, 2004). Diez et al. (2006) also reported maximum incidence during January and March. Singh and Saravanan (2008) observed that incidence increased with maximum temperature, slight rainfall and morning RH, and decreased with increasing minimum temperature, evening RH, wind velocity and sunshine hours. Nitin et al., (2018) revealed that the initial incidence of pea leaf miner, pea aphid, and stem fly was observed during last week of November to first week of December and subsequently reaching to peak on first and second week of February. Sapkal et al.

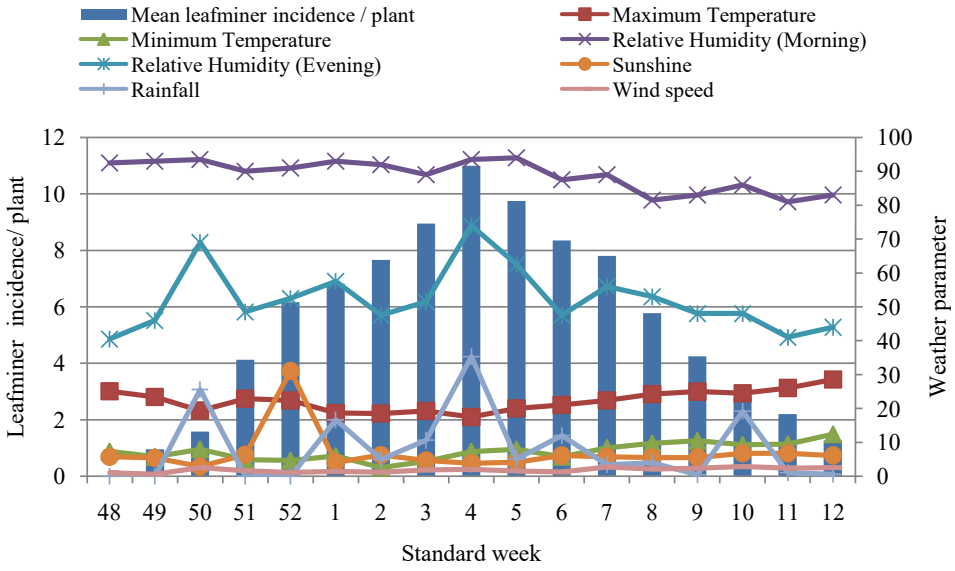


Fig. 1. Seasonal incidence of *C. horticola* on garden pea (2017 and 2018, pooled)

Table 1. Correlation coefficients - incidence of *C. horticola* vs. weather parameters (2017, 2018, pooled)

Incidence	Temperature (◦C)		Relative humidity (%)		Sunshine	Rainfall	Wind speed
	Maximum	Minimum	Morning	Evening	(hr)	(mm)	(km/ hr)
	-0.714**	-0.385	0.258	0.544*	0.013	0.356	-0.074
Regression equation				Correlation coefficient (r)	R^2	Coefficient of variation (%)	
$Y=77.294 - 1.802 X_1 + 0.646 X_2 - 0.404 X_3 + 0.008 X_4 + 0.105 X_5 - 0.127 X_6 - 0.457 X_7$				0.857	0.734	73.40	

*p = 0.05; **p= 0.01; Y= Mean leaf miner/ plant, X_1= Maximum temperature, X_2= Minimum temperature, X_3 = RH % (Morning), X_4 = RH % (Evening), X_5 = Sunshine, X_6 = Rainfall and X_7 = Wind speed kmh^{-1}.

(2018) observed the seasonal incidence of *T. absoluta* with maximum incidence 41st SMW.

The incidence was with a highly significant negative correlation (-0.714) with maximum temperature, positive correlation (0.54) with evening RH; while a negative correlation was observed with minimum temperature (-0.385) and wind speed (-0.074). The multiple linear regression equations for incidence was $Y= 77.294 - 1.80X_1 + 0.646 X_2 - 0.404 X_3 + 0.008 X_4 + 0.105 X_5 - 0.127 X_6 - 0.457 X_7$. The corresponding correlation co-efficients of multiple determination (R^2) value worked out was 0.734 (p=0.05). Chaudhuri and Senapati (2004) had also reported that temperature and rainfall showed significantly positive correlation while a non-significant positive correlation was observed with RH, temperature but RH gradient showed significantly negative one. Naga et al. (2020) reported that the leaf miner reached peak during December to January and declined thereafter; also observed that number of mines, leaf infestation and number of larvae were negatively correlated with minimum temperature (-0.61**), evening RH (-0.34*). The overall impact of weather factors on population buildup was 73.40 % (Table 1).

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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BIOLOGY OF *PLATYNASPIS SAUNDERSI* (COLEOPTERA: COCCINELLIDAE)

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ABSTRACT

Biology of *Platynaspis saundersi* (Crotch) was studied under laboratory conditions at the Department of Entomology, Dr YSP University of Horticulture and Forestry, Himachal Pradesh, India. The mean preoviposition period was 7.00± 0.21 days, while the oviposition period was 43.50± 0.35 days. The female lays 130.30± 5.96 eggs and the hatching was 86.32± 3.12%, with incubation period being 5.0± 0.47 days and the larval period was for 12.40± 0.35 days. The observed prepupal and pupal periods were 1.50± 0.48 and 7.50± 0.21 day, with longevity of male and female being 70.00± 2.00 and 79.00± 0.89 days, respectively.

Key words: *Plantynaspis saundersi*, *Solanum nigrum*, biology, egg, hatching, oviposition, longevity, prepupal period, male, female, total developmental period

Ladybird beetles have great economic importance as their larval and adult stages are predaceous on important crop pests such as coccids and other soft-bodied insects including aphids (Hippa et al., 1978; Kring et al., 1985). Species of the genus *Platynaspis* feed on aphids, and is found in association with those insects infesting bean, sorghum, tobacco, cotton, maize, potato, Lathyrus, soybean, *Solanum nigrum*, chili, sweet potato, lentil, mustard, brinjal, groundnut, sunflower and cabbage crop (Gautam et al., 1995; Duffieded,1995). Bean aphid *Aphis craccivora* Koch attacks the bean plant and leguminous crops (Srivastava and Singh, 1986), resulting in 20-40 % yield loss (Islam, 2007). In India, bean growers use various insecticides to control aphids, but these leave residues over the sprayed surface. The random use of pesticides causes phytotoxicity and destruction of beneficial organisms such as parasitoids, microorganisms, predators and pollinators (Luckman and Metcalf, 1978). These have led to compatible pest management programs Hodek (1970). One of these is biocontrol with predators, of which coccinellids are important. The study of biology of *Platynaspis. saundersi* would help in using such predators of black aphid towards biocontrol, and hence the present study.

MATERIALS AND METHODS

The experiment was conducted at the laboratory of the Department of Entomology, Solan, Himachal Pradesh, India, from April 2018 to May of 2019 (25.02± 4.5 ºC, 72.05 ± 0.95% RH), and at at the Biocontrol Laboratory at Nauni during April to May 2018 and 2019. A culture of *P. saundersi* was established with males and females collected by sweep net from the unsprayed horticulture field and reared in petridishes on *Solanum nigrum.* Aphids were also collected on a daily basis with infested *S. nigrum* leaves, twigs, stems and inflorescences from unsprayed fields and supplied as food. These beetles were sexed and paired in petridishes (6x 1 cm) having bottom covered with blotting paper (Whitman filter paper no1). After hatching of eggs, the grubs were transferred to petri dishes (11cm dia) and reared on aphids. The emerged adults were sexed and confined in pairs in petridishes and fed on aphids. Egg masses were collected and reared, and thus continued for obtaining a large number of larvae and adults. Eggs laid/ female during 24 hr was counted and thee kept in separate petridish to determine the eggs laid/ female, hatching period and (%); after hatching young larvae were transferred individually in petri dishes (6 cm dia) containing blotting paper at the bottom. Fresh *S. nigrum* shoots infested with aphids were provided as food, and larvae were observed twice daily at 12 hr interval until pupation. The number of instars and period of each instar were recorded. The pupae were kept undisturbed until the emergence of the adult, and pupal period was recorded with ten replications. Data were analysed for ANOVA and the mean values separated by Duncan's Multiple Range Test (DMRT) using SPSS, version 12.01 (SPSS, Chicago, IL, USA) and cross checked by ICAR GOA developed software WASP1.

RESULTS AND DISCUSSION

The time between the date of adult emergence and the first egg deposition was considered as the preoviposition period, and for *P. saundersi* it was 7.00± 0.21 days, which is similar to the results of Agarwala et al., (1988) as 6 to 10.33 days on *A. craccivora.* The oviposition period, the duration between first and last egg-laying was 43.50± 0.35 days. The incubation period was 5.0± 0.47 days, and Chowdhury et al., (2008) reported that it was 2 and 4 days, respectively using bean aphids as host. The total larval period (1st instar to 4th instar) was 12.40± 0.35 days. The number of eggs laid/ female was 130.30± 5.96, while the hatching was 115.40± 6.07 with % hatching being 86.32± 3.12%. Sakurai et al. (1991) reported that the quality of food and environmental factors like temperature, humidity also play an important role on the biology of coccinellid beetles. The newly hatched larva lasted for 1.82± 0.21 days; later instars lasted 3.21± 0.89, 4.20± 0.48, and 4.89± 0.32 days. The prepupal and pupal periods were 1.50± 0.48 and 7.50± 0.21 days, The number of eggs laid/ female was 130.30± 5.96, with longevity of male and female being 70.00± 2.00 and 79.00± 0.89 days. Ngammuang (1987) found the longevity of male and female as 37.8± 15.24 and 59.53± 23.53 days when fed on *A. craccivora.* The total developmental period was 77.41± 1.46 days.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

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EFFICACY OF INSECTICIDES AGAINST SESAME SHOOT WEBBER *ANTIGASTRA CATALAUNALIS*

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ABSTRACT

Field experiments evaluated the efficacy of insecticides against sesamum shoot webber *Antigastra catalaunalis* Duponchel. Emamectin benzoate 5 SG @ 200g/ ha recorded minimum damage (1.93% and 1.31%) and larval incidence (1.87 and 2.70/ plant) during kharif and rabi, respectively. Predatory coccinellids and spiders (0.61 and 0.68/ plant), maximum seed yield of 650 kg/ ha and highest B: C ratio of 1:2.49 were obtained with emamectin benzoate 5 SG.

Key words: *Antigastra catalaunalis*, capsule borer, TMV 4, emamectin benzoate, coccinellids, spiders, yield, cost benefits

Sesame (*Sesamum indicum* L.) is an important oilseed crop with sesame oil being a high priced oil (Alegbejo et al., 2003). Sesame yield is relatively low in India, and it is partly due to insect pests and diseases. In India, it is grown in many states including Tamil Nadu (Sheeba and Indiragandhi 2020; 2021), and its oil has medicinal value (Gnanasekaran et al., 2010). Sesame quality and quantity gets affected due to insect pests as it is attacked by more than 65 insect pests. Of these, the leaf/ shoot webber cum capsule borer *Antigastra catalaunalis* Duponchel (Lepidoptera: Pyraustidae) is the most serious reducing the yield considerably (Sheeba and Indiragandhi 2020; 2021; Ahuja, 1990; Ahuja and Kalyan, 2002). In India, *Antigastra* damage during flowering stage and capsule formation stages respectively will cause yield loss about 2-75% and 1.4 to 31.2% and when it reached 10% ETL, immediately spraying operations are to be done (Egonyu et al., 2005; Singh, 1987). This study provides the results of field experiments carried out to evaluate the efficacy of some insecticides.

MATERIALS AND METHODS

Field experiments were conducted during two seasons viz., kharif (July 2018) and rabi (February 2019) at the Oilseeds Research Station, Tindivanam ($12^0$12'30''N & $79^0$40'17''E), Tamil Nadu, India. The experiments were laid out in a randomized block design (RBD) with seven treatments replicated four times, with variety TMV 4 sown in a plot size of 5 x 4 m^2 with 30x 10 cm spacing. The treatment evaluated include- spinosad 45SC @ 200 ml/ ha, fipronil 5SC @ 1000 ml/ ha, emamectin benzoate 5SG @ 200g/ ha, chlorpyriphos 20EC @ 1250 ml/ ha, profenophos 50EC @ 1000 ml/ ha, quinalphos 25EC @ 750 ml/ ha and untreated check. The package of practices except plant protection measures was followed. The spraying of insecticides was given on 5th August, 2018 and 3rd March, 2019 when the incidence of shoot webber noticed during kharif and rabi, respectively. Hand operated high volume knapsack sprayer was used, with spraying done in the late evening. The observations on *Antigastra* damage in plants and larvae/ plant were recorded one day before and 1, 3 and 7 days after spray; damage was calculated as per the methodology described by Khan et al. (2009). The larval counts were recorded in 10 randomly selected plants/ plot. During harvesting seed yield was computed to calculate the cost economics. The data were analyzed statistically following the Gomez and Gomez (1984), with treatment means compared by Duncan's Multiplication Range Test (DMRT) (Duncan, 1951).

RESULTS AND DISCUSSION

Results of the kharif trials indicated that least plant damage (3.25, 2.60, 1.93%) was observed with emamectin benzoate 5SG @ 200g/ ha; also with minimum number of larvae of 3.24, 2.40, 1.87/ plant. The next best treatments were spinosad 45SC and fipronil 5SC, with % reduction over control with emamectin benzoate being 66.67% (Table 1). Yalawar et al. (2020) reported that emamectin benzoate 5%

Table 1. Effect of insecticides on *A. catalaunalis*, its natural enemies, yield and CBR

Kharif season

Treatment	Dosage ml/ g ha^{-1}	Plant damage (%)				Larvae/ plant				Damage ROC (7 DAS)	Natural enemies (no/ plat)		Yield (kg ha^{-1})	Increase over control	CBR
		PTC	1 DAS	3 DAS	7 DAS	PTC	1 DAS	3 DAS	7 DAS		Coccinellid	Spider			
Spinosad 45SC	200	4.12 (17.00)	3.55^{bc} (12.60)	2.84^{b} (8.10)	2.15^{a} (8.43)	3.86 (14.90)	3.39^{b} (11.50)	2.73^{b} (7.50)	2.00^{ab} (4.00)	47.44	0.45	0.62	575^{b}	47.44	2.26
Fipronyl 5SC	1000	4.23 (17.90)	3.91^{c} (15.30)	2.88^{b} (8.30)	2.2^{a} (8.53)	3.80 (14.50)	3.33^{ab} (11.10)	2.70^{b} (7.30)	2.36^{b} (5.60)	53.85	0.34	0.50	600^{b}	53.85	2.32
Emamectin benzoate 5SG	200	4.00 (16.00)	3.25^{a} (10.59)	2.60^{a} (6.80)	1.93^{a} (7.98)	3.74 (14.00)	3.24^{a} (10.50)	2.40^{a} (5.80)	1.87^{a} (3.50)	66.67	0.61	0.68	650^{a}	66.67	2.49
Chlorpyriphos 20EC	1250	4.01 (16.10)	3.34^{ab} (11.20)	2.72^{ab} (7.40)	2.54^{a} (9.19)	3.92 (15.40)	3.67^{bc} (13.50)	2.81^{b} (7.90)	2.42^{ab} (5.90)	35.90	0.50	0.46	530^{bc}	35.90	2.18
Profenophos 50EC	1000	4.05 (16.40)	3.39^{ab} (11.50)	2.81^{b} (7.90)	2.55^{a} (9.19)	4.07 (16.60)	3.55^{c} (12.60)	2.86^{c} (8.20)	2.49^{ab} (6.20)	32.05	0.44	0.48	515^{bc}	32.05	2.16
Quinolphos 25EC	750	4.09 (16.80)	3.47^{b} (12.10)	2.82^{b} (8.00)	2.6^{a} (9.28)	4.20 (17.70)	3.70^{c} (13.70)	2.96^{c} (8.80)	2.53^{c} (6.40)	21.79	0.32	0.53	475^{c}	21.79	1.99
Control	-	4.30 (18.50)	4.83^{d} (23.40)	5.20^{c} (27.90)	6.27^{b} (14.51)	4.44 (19.75)	4.80^{c} (23.10)	5.17^{d} (26.80)	5.45^{d} (29.70)	-	1.2	0.85	390^{d}	-	-
SEd	-	0.67	0.44	0.37	0.94	0.37	0.43	0.37	0.34	-	-	-	21.3	-	-
CD (p=0.05)	-	NS	0.96	0.81	1.97	NS	0.94	0.80	0.74	-	-	-	46.3	-	-

Rabi season

Treatment	Dosage ml/ g ha^{-1}	Plant damage (%)				Larvae/ plant				Damage ROC (%) 7DAS	Natural enemies population (no/ plant)		Yield (kg ha^{-1})	Increase over control (%)	CBR
		PTC	1 DAT	3 DAT	7 DAT	PTC	1 DAT	3 DAT	7 DAT		Coccinellid	Spider			
Spinosad 45SC	200	4.15 (17.20)	3.61^{ab} (13.00)	3.10^{ab} (9.60)	2.88^{a} (9.76)	4.38 (19.20)	3.55^{b} (12.60)	2.89^{ab} (8.30)	2.59^{b} (6.29)	46.84	0.48	0.64	580^{b}	46.84	2.29
Fipronyl 5Sc	1000	4.05 (16.40)	3.58^{ab} (12.50)	2.83^{ab} (8.05)	2.74^{a} (9.55)	4.07 (16.60)	3.38^{ab} (11.50)	2.70^{ab} (7.40)	2.00^{a} (4.00)	50.63	0.35	0.56	595^{a}	50.63	2.30
Emamectin benzoate 5SG	200	4.07 (16.60)	3.36^{a} (11.29)	2.70^{a} (7.30)	2.31^{a} (8.72)	3.92 (15.40)	3.25^{a} (10.59)	2.41^{a} (5.80)	1.70^{a} (3.38)	62.03	0.56	0.65	640^{a}	62.03	2.38
Chlorpyriphos 20EC	1250	4.34 (18.80)	3.47^{a} (12.10)	3.25^{ab} (10.59)	2.95^{a} (9.89)	4.38 (19.20)	4.11^{c} (16.90)	3.69^{bc} (13.60)	2.70^{b} (7.30)	37.97	0.52	0.58	545^{b}	37.97	2.25
Profenophos 50EC	1000	4.29 (18.40)	3.93^{ab} (15.40)	3.60^{bc} (12.90)	3.21^{a} (10.30)	4.11 (16.90)	3.83^{b} (14.70)	3.33^{bc} (11.10)	3.29^{c} (10.80)	34.18	0.45	0.42	530^{bc}	34.18	2.20
Quinolphos 25EC	750	4.34 (18.80)	4.12^{bc} (16.92)	3.70^{bc} (13.69)	3.36^{a} (10.55)	4.20 (17.70)	3.75^{b} (13.90)	3.55^{bc} (12.60)	3.24^{c} (10.50)	27.85	0.46	0.58	505^{bc}	27.85	2.15
Control	-	4.30 (18.50)	5.18^{d} (26.80)	5.44^{d} (29.60)	6.63^{b} (14.92)	4.44 (19.75)	5.20^{d} (24.00)	5.45^{d} (29.70)	5.57^{d} (29.90)	-	0.92	0.96	395^{c}	-	-
SEd	-	0.44	0.43	0.44	0.51	0.27	0.14	0.27	0.29	-	-	-	22.9	-	-
CD (p=0.05)	-	NS	0.91	0.93	1.06	NS	0.30	0.57	0.61	-	-	-	48.5	-	-

PTC- Pretreatment count; Values mean of three replications; Values in parantheses square root transformed, for population and arc sine transformed for % damage; ROC – Reduction over control. PTC- Pre treatment count. Values mean of three replications. Values in parantheses square root transformed for population and arc sine transformed for damage.

SG was the most effective. During rabi season also emamectin benzoate was superior with 3.36, 2.70 and 2.31% damage, respectively on 1,3,7 DAS (Table 1); the next best were fipronil 5SC and spinosad 45SC, and on par with each other; similarly emamectin benzoate 5SG @ 200g/ ha treated plots recorded the least larval counts. Maximum reduction over control (62.03) was observed in emamectin benzoate 5SG followed by fipronil 5SC (50.63) and spinosad 45SC (46.84). Results of the present study agree with those on emamectin benzoate 5% SG and spinosad 45%SC (Yalawar et al., 2020). Emamectin benzoate stood first in reducing damage (%), larval incidence (no/ plant), increased yield and economic returns in two consecutive seasons. The natural enemies viz., coccinellids (0.61) and spiders (0.68) were more in emamectin benzoate 5SG. Jyoti and Basavana (2008) observed that emamectin benzoate 5SG and spinosad 45SC were safe to natural enemies. *Schymnus* spp., and *Coccinella undecimpunctata* are not negatively affected by emamectin benzoate (Sechser et al., 2003). Maximum yield was obtained with emamectin benzoate 5SG (640-650 kg/ ha) (Table 1), with maximum of cost benefit ratio (2.38 -2.49). Increased yield in emamectin benzoate treated plots might be due to the reduced damage. Varma et al. (2013) reported that emamectin benzoate 0.001% treated plots recorded minimum flower damage.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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PRIMARY AND SHADOW ENHANCERS OF DORSAL - VENTRAL PATTERNING GENES IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Recent studies have revealed that multiple enhancers can activate the transcription of a gene. They can be classified as primary or shadow enhancers. Earlier studies suggested that many dorsal target genes of *Drosophila melanogaster* might be regulated by these shadow enhancers. Therefore, primary and shadow enhancers of five different dorsal targeting genes viz., *zen, sim, vnd, bun,* and *rho* were predicted in this study. In addition, novel cluster motifs with high strand scores have been predicted and are referred to as primary enhancers, whereas similar sequences within 5kb are referred to as shadow enhancers. The information generated from this study may lead to the evolutionary innovation of enhancer mechanisms.

Key words: *Drosophila melanogaster*, enhancers, transcription factors, in-silico, gene expression, activator, repressor, flanking sequence, cluster motifs

In the world of insects, *Drosophila* has been studied widely for genetic research as an ideal model for understanding how these variations and regulations contribute to its development. The gene regulatory mechanism of controlling dorsal-ventral axis formation in insects has undergone drastic changes in the field of evolution (Kapil and Kaur, 2021). This process in the *Drosophila* is mainly regulated by the dorsal protein which establishes different expression patterns in dorsal-ventral patterning by binding or activating different transcription factors. Dorsal activates twist protein in the mesodermal region and these two maternal factors further activate snail that limits the expression of dorsal in the mesodermal region. (Jiang et al., 1991). These three binding factors bind in the cluster to activate different sets of the gene (Ip et al., 1992; Zeitlinger et al., 2007). The Suppressor of hairless (Su (H)) and Zelda (vfl) also play important role in the expression of the DV patterning genes (Jiang and Levine, 1993). Both the transcription factors control the positioning of the dorsal gene boundary of the DV axis (Ozdemir et al., 2014).

Earlier, studies observed that in addition to a primary enhancer that is well defined, many genes have secondary elements with very similar transcription factor binding clusters (Hong et al., 2008; Scholes et al., 2019; Waymack et al., 2020). These secondary factors or elements are known as shadow enhancers. Shadow enhancers overlap the activity of primary enhancers and arise from duplication and separation of protein-coding sequences. Genetic repetition has long been observed as a prospective source of evolutionary alteration (Krischner and Grehart, 1998). These provide sturdiness genetic variation of a population, allow them to develop undisturbed (Cannavo et al., 2015). In vitro studies have revealed that many dorsal target genes are regulated by primary and shadow enhancers even when they are located far away from the target genes (Hong et al., 2008; Guerrero et al., 2010; Small and Arnosti, 2020). However, only a few of the shadow enhancers were predicted to be located on the flanking regions of the genes. Indeed, it is necessary to study these primary and shadow enhancers in flanking regions of target genes for understanding evolving novel expression patterns. Hence the current study was designed to predict the locations of primary and shadow enhancers of dorsal-ventral patterning genes (*Zerknullt, Single-minded, Ventral nervous system defective, Bunched* and *Rhomboid*) of *Drosophila* and the clusters of TFs (dorsal, twist, snail, Zelda, and Suppressor of hairless) by the use of computational methods to understand the evolution in enhancers mechanism. This study attempts to look into the primary and shadow enhancers in the 20 kb flanking regions of the target genes using in silico approach.

MATERIALS AND METHODS

Gene Sequences were obtained in FASTA format from Flybase version FB2019_06. Genes are symbolized by their FlyBase ID as shown in Table. These sequences were obtained and modified in the year of 2019-2020. These sequences are modified in 20kb flanking regions through BLAST tool. The binding motif sequences of

dorsal, twist, snail, Zelda, and Suppressor of hairless were obtained from the JASPAR database. It is an open-access database that contains transcription factor binding profiles as position frequency matrices (PFMs) and TF flexible models (TFFMs) for TFs across multiple species in six taxonomic groups (Khan et al., 2018). Flanking gene sequences and motifs were uploaded to the Cluster- Buster software to predict binding sites and binding site clusters. The nucleotide sequence patterns, motifs preferentially bound by different transcription factors have been collected in this database (Martin et al., 2003). This is used for finding different clusters for pre-specified motifs in DNA sequences. In this database, the gene and motif sequences were submitted and results showed the location of the gene, size of predicted enhancer (primary and shadow), and binding sites. We arbitrarily refer to the newly identified enhancer as the shadow enhancer having similar binding sites but with a low strand score as the primary enhancer with a strong strand score of transcription factor binding clusters.

RESULTS AND DISCUSSION

In this study, the location of primary and shadow enhancers, size of the enhancer, binding sites clusters of different transcription factors have been analyzed. In order to predict primary and shadow enhancers, similar clusters of transcription factor binding sites (TFBS) on target genes were identified using Cluster-Buster tool. The target gene sequences were collected in FASTA format from Flybase version FB2019. Using the BLAST tool, these gene sequences were modified into a 20kb flanking region. PWM, transcription factor binding motifs format were derived from JASPAR. These 20kb flanking sequences and PWM for motifs were uploaded into the Cluster-Buster software version of year 2019-2020 for the identification of TFBS. The generated output data is summarized in Table 1. In this study, the *zen* gene showed the binding sites for twi, snail, and dl factors. Both the primary and shadow enhancers were located downstream to the TSS of the gene as depicted in Fig. 1. The size of the predicted primary enhancer was found to be 487 bp and that of the shadow enhancer was 181 bp. The *sim* gene showed binding sites for dl, twi, vfl, Su (H), and snail. The size of the predicted primary enhancer was 340 bp and that of the shadow enhancer was 245 bp. Both the primary and shadow enhancers were located downstream to the TSS of the gene as shown in Fig. 2. Apart from the three known binding sites (dl, twi, sna), this study has identified two more binding sites *viz*. Su (H) and vfl which further controls the expression of the *sim* gene. The binding of Su(H) up-regulates sim expression in the mesectoderm and prevents the uprooted expression of the sim gene dorsally in the neuroectodermal region (Morel and Schwiesguth, 2000). *Vnd* gene showed the binding sites for dl, snail, and twi transcription factors. The size of the predicted primary enhancer was 756bp and that of the shadow enhancer was 187 bp.

The primary enhancer was located downstream of the TSS whereas the shadow enhancer was lying downstream just near to the TSS (Fig. 3). *Bun* gene showed the binding sites for dl, twist, and snail. The size of the primary enhancer was 400 bp and the size of the secondary enhancer was 412 bp. Both the enhancers were lying downstream of the TSS (Fig. 4). In the current study, the *Rho* gene showed binding sites for dl, twi, snail, and vfl. The primary enhancer was 544 bp in size and the secondary enhancer was 150 bp in size. Both predicted enhancers located upstream of the TSS as shown in Fig. 5. This study has identified primary and shadow enhancers of five different dorsal targeting genes viz. *zen, vnd, rho, sim*, and *bun*. To validate this prediction, primary enhancer sequences were aligned with verified enhancers in the Redfly

Table 1. Gene sequences, Flybase IDs, used for enhancer prediction, and size of the enhancer

Gene	Gene Sequence	Flybase ID	TSS	Location of enhancer from TSS of the gene		Size of the enhancer		Figure no.
				Primary	Shadow	Primary	Shadow	
Zerknullt (zen)	6752864-6754194	FBgn004053	6752864	+	+	487bp	181bp	1
Single minded (sim)	13057755-13078124	FBgn0004666	13057755	+	+	340bp	245bp	2
Ventral nervous system defective (vnd)	582121-598151	FBgn0261930	582121	+	+	756bp	187bp	3
Bunched (bun)	12433118-12528775	FBgn0259176	12455540	–	–	400bp	412bp	4
Rhomboid (rho)	1463811-1468944	FBgn0004635	1463811	–	–	544bp	150bp	5

The enhancers' locations denoted by "+" (downstream to the TSS) and "-" (upstream to the TSS); Table also depicts figure numbers in which results generated by cluster buster software are summarized in form of 2-D diagrams.

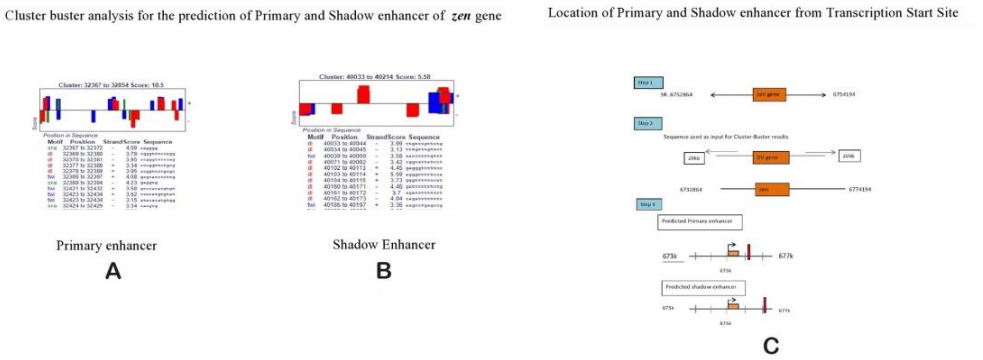


Fig. 1. Screenshot of Cluster – Buster output and 2- D construct for the location of Primary and shadow enhancers of the *zen* gene. Both enhancers were located downstream to the TSS

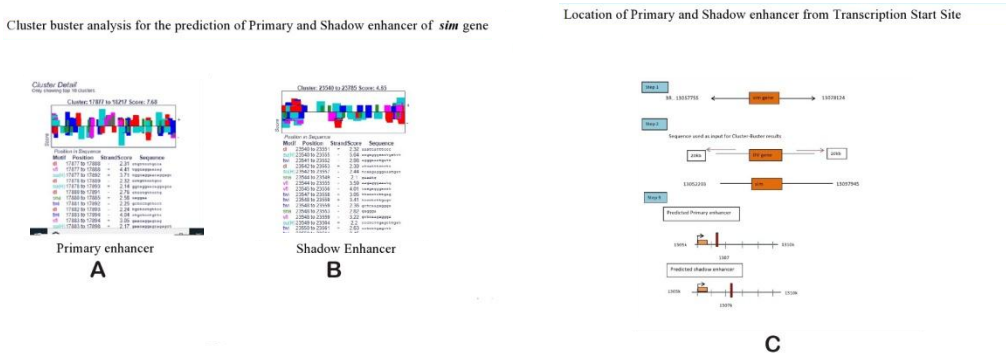


Fig. 2. Location of primary and shadow enhancers of the *sim* gene. Both the primary and shadow enhancers were located downstream to the TSS

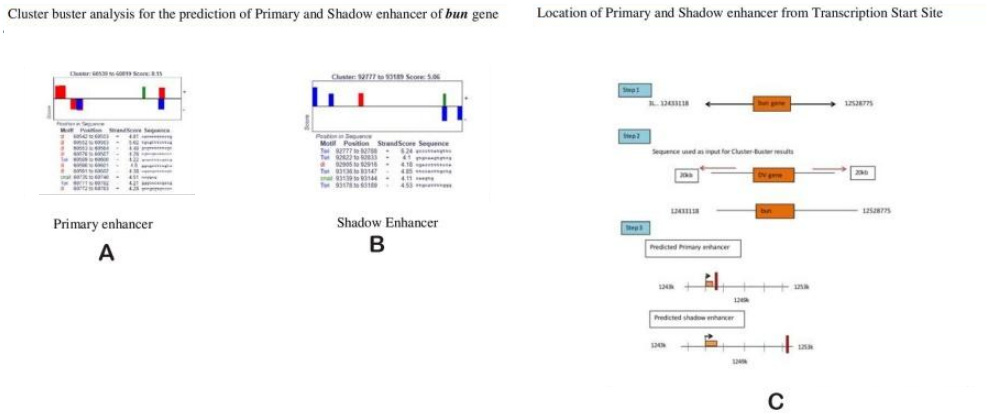


Fig. 3. Cluster- buster output and construct for the *vnd* gene shows that the primary enhancer was located downstream of the TSS whereas the shadow enhancer was lying downstream and just near the TSS

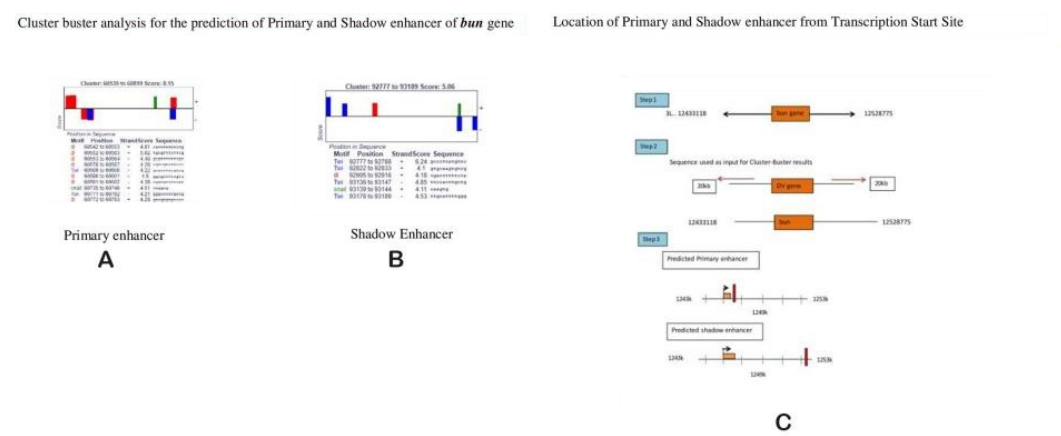


Fig. 4. 2- D construct and Cluster- Buster result for the location of Primary and shadow enhancers of *bun* gene. Both the enhancers were lying downstream of the TSS

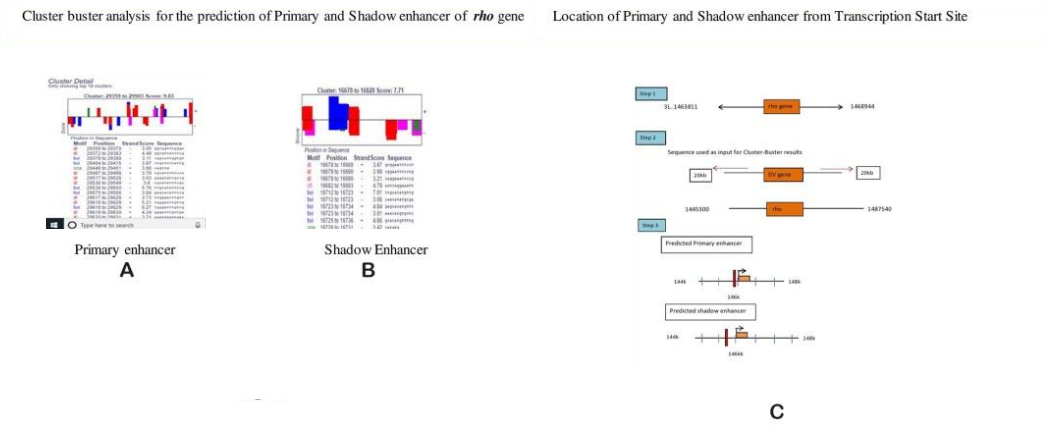


Fig. 5. This figure is showing that both predicted enhancers of the *rho* were located upstream of the TSS

database. In relation to previous studies, this study predicted multiple enhancers for all selected dorsal target genes. Apart from the prediction of previously identified multiple enhancer, this study has predicted new shadow enhancer sequences and strong primary enhancers with respect to their postion in the flanking regions of the target DV genes. Therefore, it can be concluded that gene expressions can be controlled by primary and shadow enhancers and these can be located far away from the target gene. The study of these enhancers and their evolution will further help to understand variations in regulatory elements.

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AUTHOR CONTRIBUTION STATEMENT

TK conceived and designed research. SK has predicted data. TK and SK analyzed data. SK wrote the manuscript. Both authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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DIAGNOSTICS OF *PERINA NUDA* (F) (LEPIDOPTERA: EREBIDAE)

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ABSTRACT

Clearwing tussock moths were collected from different parts of Tamil Nadu, India. The occurrence of *Perina nuda* (F.) has been documented with morphological studies. Male moths bear no resemblance to females, with almost hyaline forewing and hind wings drab grey to pale purplish grey with a hyaline patch at the apical angle of the hind wing. Females with capucine buff-coloured wings. Males have asymmetrical genitalia with pointed-finger shaped uncus. The species identity was confirmed through DNA barcodes submitted to GenBank (RKB004-21 and MZ540881). The molecular phylogeny revealed that it formed separate clade from the available *P. nuda* barcodes with a meagre 2% variation.

Key words: Clearwing tussock moth, *Perina nuda*, redescription, morphology, genitalia, diagnosis, DNA barcoding, phylogeny

Genus *Perina* was established by Walker (1855) with type species *basalis* described from Nepal and with distribution records from North India and Hong Kong under family Psychidae (Walker, 1855). Earlier, *Bombyx nuda* was described from India (Fabricius, 1787) and was revised under Liparidae (Kirby, 1892) and now classified under *Perina* of family Lymantriidae (Watson et al., 1980) while *Perina basalis* is considered to be the junior subjective synonym of *P. nuda* (Beccaloni et al., 2003). At present, Lymantriidae has been subordinated as a subfamily of Erebidae (Zahiri et al., 2011) and hence, genus *Perina* is now classified under Leucomini (Holloway, 1999) of Lymantriinae of family Erebidae. Genus *Perina* comprises of six species viz*., Perina nuda* (Fabricius, 1787) and *P. pura* Walker, 1869 with distribution records from India (Hampson, 1893; Kaleka, 2010; Smetacek, 2008) and *P. kalisi* Collenette, 1949*, P. lodra* Moore, 1859*, P. psamma* Collenette, 1933 and *P. sunda* Holloway, 1999 from elsewhere (Holloway, 1999). Except for a brief description of Hampson (1893) and distributions records of *Perina* spp. across India, the information is scanty. Perusal of literature indicated that genitalia and molecular details from India are wanting. Owing to morphological similarity and sympatry among various *Perina* sp., proper identification of species is imminent. This study characterizes *Perina nuda* from Tamil Nadu as the occurrence of *P. pura* from peninsular part of India remains to be documented.

MATERIALS AND METHODS

Light trap collections of moths from different parts of Tamil Nadu was undertaken viz*.,* Kodaikanal (10°22'N, 77°45'E, 2133 masl), Thadiyankudisai (10°30'N, 77°69'E, 1100 masl), Thandikudi (10°27'N, 77°60'E, 1500 masl) of Lower Pulney hills of the Western Ghats, Jawadi hills (12°58'N, 78°83'E, 857 masl) of the Eastern Ghats and from the plains of Yethapur (11°65'N, 78°47'E, 282 masl) (Salem district), Aiyyur (10°27'N, 77°71'E, 1060 masl) (Krishnagiri district), Coimbatore (11°01'N, 76°94'E, 425 masl) and Pollachi (10°64'N, 76°88'E, 293 masl) (Coimbatore district). The collected specimens were deposited in the TNAU Insect Museum, Coimbatore. Specimens collected under Network Project on Insect Biosystematics (NPIB), TNAU Insect Museum (IM) Project and students' collection of Tamil Nadu Agricultural University, Coimbatore were also examined. Taxonomic characters viz*.,* antennae, labial palp, fore wing (FW), hind wing (HW), legs and female (♀), male (♂) genitalia were examined. Colours of adult moths were described as per Ridgway (1912). For the study of wing venation, slides of wings were prepared as per Zimmerman (1978); wing venation description follows Comstock and Needham (1898). Genetalia study follows Robinson (1976) and terminology of Klots (1970). Specimens were examined and photographed (genitalia) under stereozoom microscope (Leica: M205A, Software LAS

V4.12) and adult moths were photographed with Digital Single Lens Reflex camera (DSLR) (Nikon D3100).

The DNA barcoding and phylogeny study of *P.nuda* adults collected from Coimbatore were undertaken. Genomic DNA was extracted from 3 legs of the fresh specimen using CTAB method (Gawel and Jarret, 1991). DNA concentration was measured using nanodrop spectrophotometer (Nanodrop One, Thermo Scientific, Madison, USA). The PCR amplification was performed in 30 µl reactions containing 15 µl of master-mix, 2.5 µl of each primer, 6 µl of DNA and 4 µl DNase free water. The amplification cycle of Hebert et al. (2003) was followed. The results were visually verified by electrophoresis on 1.5% agarose gel stained with ethidium bromide. The PCR product was sequenced through Eurofins Genomics India Pvt Ltd., Bangalore, India. Two sequences were submitted to GenBank and accession numbers were obtained (RKB004-21 and MZ540881). These sequences were compared with 12 sequences from GenBank, using Blastn in GenBank to check genetic similarity. Sequences were aligned using CLUSTALW (Thompson et al., 1997) implemented in MEGA version X (Kumar et al., 2018), with default parameters. Model-based phylogenetic analyses were performed using Neighbour-Joining (NJ) method and Bayesian inference in MEGA version X (Kumar et al., 2018) with the branch support values evaluated by 1000 bootstrap replicates under Tamura 3-parameter model with gaps/ missing data treatment as partial deletion and 95% cut-off. The sequence of *Bombyx mori* from GenBank was used as outgroup.

RESULTS AND DISCUSSION

Genus *Perina* Walker, 1855

Perina Walker, 1855: 966 (Type species: *nuda* Fabricius). *P. basalis* is a junior subjective synonym of *Bombyx nuda* Fabricius, 1787, **2**: 119.

Perina nuda (Fabricius, 1787)

Redescription: Medium sized moths with globular compound eyes; antennae bipectinate, bipectinate comb sickle shaped, with pectin long at the base and tapering towards the tip; labial palpi curved upward, second segment longest of the three; proboscis tip without any spines. Fore tibia with epiphysis; mid tibia and hind tibia with two tibial spurs. Forewing (FW) sub triangular, apical margin slightly convex, and hind wing (HW) sub triangular to squarish with apical margin moderately convex. Sexual dimorphism very prominent. The male moths bears no resemblance to female moths. Male: Head, thorax and abdomen densely clothed with drab gray to pale purplish gray to slate black scales; tip of abdomen (scales covering the genital area) clothed with orange to pinkish orange scales. Wings: Upper side and underside: FW hyaline with drab gray to pale purplish gray to slate black scales only near the humeral angle spreading towards anal margin along A1+2; HW with drab gray to pale purplish gray to slate black scales and a hyaline patch at the apical angle revealing Sc+R1 and Rs terminating at the margin. Wingspan: 38 mm. Female: Head, thorax and abdomen densely clothed with capucine buff scales. Fore- and hind wings covered by capucine buff scales. Wingspan**:** 40-42 mm (Figs. 1, 2). Wing venation: FW: Sc ends two third of FW. R1 arises from the anterior part of discal cell and ends near costal angle. Following R1, R2 arises from the corner of discal cell and ends at costal angle. Further R3 and R5 branch from a common stem while R4 branches from R3 and ends prior to R5. HW: Sc+R1 and Rs form a basal areole before diverging and terminating at the apical margin; Sc+R1 ends at costal angle, followed by Rs. Male: M1 absent. M2 and M3 stalked. Cu1 and Cu2 branch nearer M3. A1+2 and A3 arise separately from base of wing. Female: M1 branches from Rs. M2 and M3 branches from discal cell. Cu1 and Cu2 arise from mid-point of discal cell. A1+2 arises from base of wing. A3 either inconspicuous or absent.

Male genitalia: Uncus slender, narrow, pointed finger-like and slightly left oriented; tegumen setose, shoulder on the right more prominent while the shoulder on the left less conspicuous and appear asymmetrical; saccus cylindrical small, setose on plate- like vinculum; valva linear, corona part of the valve appear slightly folded and flap-like, non-setose in the coronal area. Flap more prominent on the right side and almost indistinct on the left side, hence asymmetrical; gnathos beak-like and prominent. Aedeagus twisted and almost ‘z’ shaped. Cornutii absent. (Fig. 3 - 5). Female genitalia: Anal papillae clasp-like and setose, sub-genital plate heavily setose along the ventral side, posterior and anterior apophyses slender and equal in length, lamella post-vaginalis heavily sclerotized and forms an interior ring-like structure connecting to the apophyses, ostium almost circular and sclerotized, ductus bursae slightly sclerotized and short with ridge like longitudinal patterns, corpus bursae ovoid and bulged in the middle, signum line like, two appendix bursae globular at either lateral sides of anterior corpus bursae (Fig. 6).

Through molecular analysis it is confirmed that the collected moths are *Perina nuda* (Fig. 7). *Perina*

Perina nuda (Fabricius, 1787)



Fig. 1. Dorsal view of male adult



Fig. 2. Dorsal view of female adult

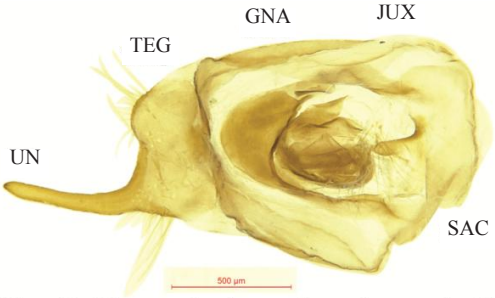


Fig. 3. Ventral view of male genitalia



Fig. 5. Aedeagus

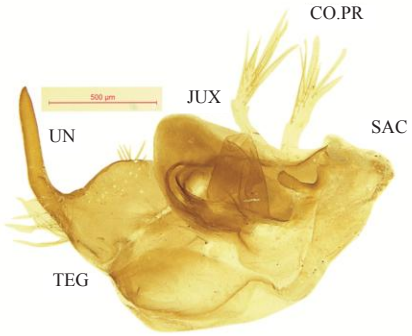


Fig. 4. Lateral view of male genitalia

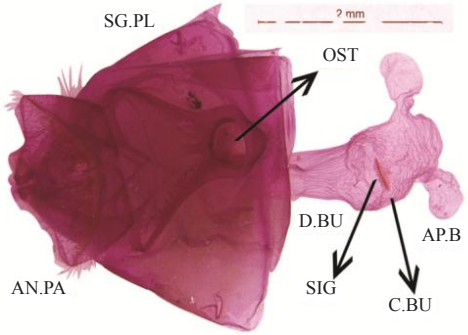


Fig. 6. Dorsal view of female genitalia

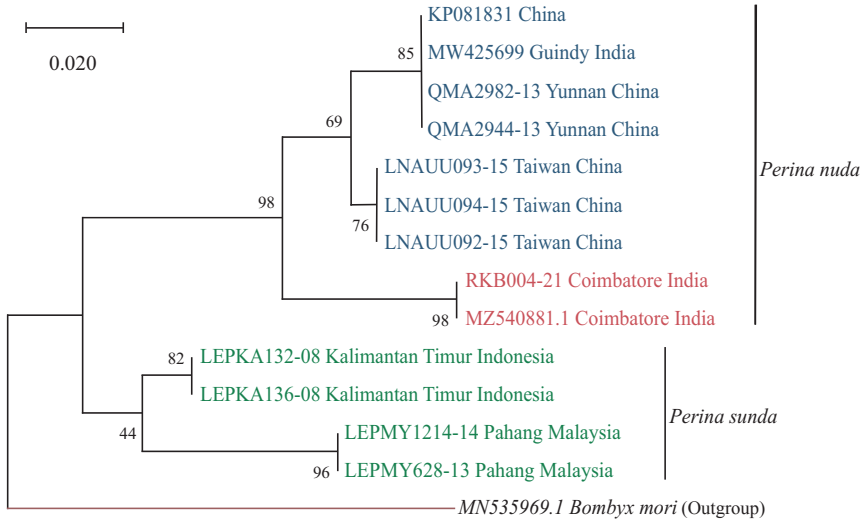


Fig. 7. Phylogenetic tree of *Perina nuda* and *P. sunda*

nuda forms a separate clade / branch from that of *P. sunda*. Within the clades the species group together on geographical basis with the exception of MW425699 Guindy population (Sayers et al., 2021; Ratnasingham and Hebert, 2013).

Remarks: The most comprehensive study focused on phylogenetic relationships of Lymantriinae (Wang et al., 2015) included only *P. nuda* in their analyses. We find that the branch length of Coimbatore population to be longer than the rest which means they have high molecular evolution. Even among *P. sunda*, the Pahang, Malaysia population has a longer branch than that of Kalimantan Timur, Indonesia population. The Guindy population branch is on par with that of Yunnan, China population but this deviation needs confirmation through further sampling and analyses (Sayers, 2021). Sexual dimorphism is common among the species of *Perina*. It is evident that sexual dimorphism is very prominent in other members of Lymantriinae. For instance, it has been observed in *Euproctis dimorphissima* (Holloway, 1979) and *Numenes* spp. (Inoue, 1975). Female moths of *Perina* spp. look akin to moths of tribe Nygmiini (Holloway, 1999). Male moths of *P. kalisi, P. lodra* and *P. psamma* are almost identical to *Perina nuda* (Collenette, 1949; Holloway, 1999). Generally, it is observed that Lymantriinae are pleisomorphic with quadrifid hindwing venation (Ferguson, 1978). In addition, the absence of M1in the HW of male moths as in the present study is a characteristic feature for the genus *Perina* (Holloway, 1999; Kishida, 2011).

In Leucomini, the male genitalia are asymmetrical in all three genera viz*., Perina, Leucoma* and *Dendrophleps* (Holloway, 1999). *Perina nuda* (=*subtincta* Walker) collected in East Indies is speculated to be either from India or Sundaland. The valves have been described to be slender with bulbous rod at the apex (Holloway, 1999). However, in present study, valves are linear with flaps and asymmetrical. In male genitalia, valves of *P. kalisi* and *P. nuda* resemble each other having prominent long right valve and short left valve with pointed right valve in *P. kalisi* and blunt flap-like in *P. nuda*. The female genitalia of *P. nuda* identical as described by Kishida (2011).

Host records: *Artocarpus heterophyllus* (Butani and Jotwani, 1984; Rajkumar et al., 2018; Sharma et al., 2008), *A. integrifolia* (Lefroy, 1909), *Ficus altisimma* (Cheanban et al., 2017), *F. carica* (Fletcher, 1917), *F. benghalensis* (Cheanban et al., 2017), *F. benjamina* (Cheanban et al., 2017; Meena et al., 2018), *F. microcarpa* (Easton and Pun, 1996), *F. racemosa*, *F. religiosa*, *F. retusa*, *F. rumphii* (Cheanban et al., 2017), *Ficus* sp. (Cheanban et al., 2017; Fletcher, 1919), *Mangifera indica* (Fletcher, 1919) and *Sapindus trifoliatus* (Ghorpade and Patil, 1991).

Distribution: Madhya Pradesh (Chandra and Nema, 2006; Khan et al., 1988; Meena et al., 2018), Maharashtra (Ghorpade and Patil, 1991; Gurule and Nikam, 2013), Assam (Arandhara et al., 2017), Chhattisgarh (Chandra and Nema, 2006), Andaman (Chandra, 1994; Chandra and Rajan, 1995), Himachal Pradesh, Jammu, Kashmir, Uttarakhand (Kaleka, 2012), Tamil Nadu (Kathirvelu et al., 2019), Karnataka (Mishra et al., 2016), throughout India (Butani and Jotwani, 1984; Cherian and Israel, 1939; Hampson, 1893; Kaleka, 2010; Lefroy, 1909; Rajkumar et al., 2018; Sharma et al., 2008), Nepal (Kishida, 1993), Vietnam (Schintlmeister, 1987), Bangkok (Cheanban et al., 2017), Japan (Matsumura, 1933), China (Easton and Pun, 1996; Kendrick, 2004; Kwok and Tai, 2016), South East Asia (Wallner and McManus, 1989), Tropical Asia (Muniappan et al., 2012), Asia (Prakash et al., 2009) and Pacific (Elevitch and Manner, 2006).

Material examined: INDIA. Tamil Nadu, Coimbatore, TNAU, 3♂ 4♀; NPIB leg; 29.xii.2006, PNFCBEF05, PNMCBEM08; 24.xi.2009, PNFCBEF02, PNMCBEM04; 03.iii.2011, PNFCBEF01, PNMCBEM03; 10.xi.2010, PNFCBEF03; 1♀; Orchard, 10.vi.2021; Balaji, RK leg; PNFCBEF07; 1♀; Pollachi, VIA, 18.xii.2019; Students coll. leg; PNFPOLF01; Dindigul, 1♀; Thandikudi, 10.xi.2010; NPIB leg; PNFTNKF01; 2♂ 2♀; HRS Thadiyankudisai, NPIB leg; 27.vi.2008, PNFTKDF06; 04.ii.2014, PNMTKDM09; Balaji, RK leg; 21.xii.2017, PNFTKDF03; 09.i.2018, PNMTKDM23; 1♀; Kodaikanal, 09.vii.2009; NPIB leg; PNFKKLF01; 1♂; Vellore, Jawadi hills, 30.x.2014; Ganeshkumar, M leg; PNMJWDM01; 1♂; Salem, Yethapur, 28.i.2009; NPIB leg; PNMYTPM01; 2♂; Krishnagiri, Aiyyur, 09.i.2007; NPIB leg; PNMAYRM01, 02. Abbreviations- (PNM-*Perina nuda* male; PNF-*Perina nuda* female; CBE-Coimbatore; TNK-Thandikudi; TKD-Thadiyankudisai; KKL-Kodaikanal; JWD-Jawadi hills; YTP-Yethapur; AYR-Aiyyur; M-male; F-female).

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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NEW RECORD OF *JAURAVIA ASSAMENSIS* KAPUR (COLEOPTERA: COCCINELLIDAE) FROM PENINSULAR INDIA

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ABSTRACT

The genus *Jauravia* (Coccinellidae: Coleoptera) is distributed in Myanmar, Sri Lanka, India and Nepal. This study documents the occurrence of *J. assamensis* Kapur on eight plants from Kolhapur and Satara, Maharashtra, India. Its distribution in Assam, Manipur and Meghalaya is known. This occurrence in the Western Ghats and peninsular India is detailed in this study along with the associated host plants, and life stages (larva, pupa and adult) in addition to its diagnostics.

Key words: Ladybird beetle, *Jauravia assamensis*, new record, Peninsular India, Western Ghats, plants, life stages, dignostics, occurrence

Motschulsky (1858) erected the genus *Jauravia* (Coccinellidae with two species viz., *Jauravia pallidula* and *Jauravia limbata*. Kapur (1946) made a taxonomic revision of this genus with about 11 species. This genus is known from Sri Lanka, Nepal and Myanmar besides northeast India. According to Poorani (2002), 15 species of this genus are known from the Indian subregion. The present study documents the occurrence of *Jauravia assamensis* Kapur from Kolhapur and Patan in Maharashtra. Thus, it is the first record from peninsular India. Earlier this species was reported from Assam (Kapur, 1961; Poorani, 2002), Meghalaya (Chakraborty and Biswas, 2002), Manipur (Chakrabarti et al., 2012) from India, and Sundarbazar, Lamjung, Nepal (Sajan et al., 2018). In the present study, its range extension, associated plants, life stages, morphology and diagnostics are illustrated and dsicussed.

MATERIALS AND METHODS

Specimens of *J. assamensis* were collected by handpicking and light trap methods. For identification, description by Kapur (1961) and Das et al. (2021) was used. Specimens preserved by both wet and dry methods were studied under LYNX LM-52 stereomicroscope, and measurements (length: head to apex of elytra, and width: between midlateral edges of elytra) were taken using ImageJ software. The abdomens were dissected in insect saline to extract genitalia and photographed with Olympus CX31RTSF compound microscope attached with HDMI digital camera in TCapture software. Images were stacked in Combine ZP software.

RESULTS AND DISCUSSION

***Jauravia assamensis* Kapur** (Coccinellidae: Coeloptera)

Diagnostics: Length: male 1.69-2.02 mm, width 1.54 -1.93 mm (male); female 2.02 -2.17 mm, width 1.69-1.98 mm (female). Body round, pale cream with kidney-shaped black markings on elytra. Markings about two-third wide as elytral length, rounded at each end and a little broader at the apical end, outer arc runs parallel to the elytral epipluron. Elytral epipluron is slightly explante. Pronotal anterior corners blunt, scutellum very small (Fig. 1a). Head, pronotum and elytra with thinly scattered punctures and short greyish pubescence. On the ventral side, the pubescence is more spaciously dispersed. Mandibles are predatory or carnivorous type and dark brown at their tips (Fig. 2a). The terminal segment of the maxillary palp is apically acuminate (Fig. 2b). Tarsi are pseudotrimerous with simple tarsal claws (Fig. 2c, d). The postcoxal line is incomplete (Fig. 1f). Male genitalia with basal plate subtriangular; penis guide elongate, uniformly wide from base to near tip, width double in the size of paramere, the tip of penis guide slightly narrowed and rounded (ventral view), the tip of penis guide in lateral view progressively narrowed and pointed; parameres cylindrical, distinctly longer than the penis guide, broad at the base and slightly expanded and rounded at the apex, an expanded portion is with long 18-20 setae; penis long, narrow, curved; except capsule and tip, the penis is uniform in width; tip narrow, pointed and

slightly curved outwardly; capsule moderately expanded with the longer outer arm than the inner hooked arm (Fig. 1 g-j). Female genitalia with cornu sickle-shaped, gradually broadened apically; coxites triangular, about two-thirds as long as wide, broad basally and narrow apically, apex dark with five long and few short setae; short styli with two long setae (Fig. k, l).

Material examined: Two males (LB324a, LB324b), two females (LB333a, LB333b), 26.x.2018, Shivaji University, Kolhapur, Coll. Priyanka Patil (hand picking); LB 342, one male; LB342, one female, 31.x.2018, Patan, Satara, Coll. Yogesh Mane (light trap) from forest habitat. Specimens are deposited in the Department of Zoology, Shivaji University, Kolhapur.

Distribution: India: Assam, Manipur, Meghalaya, Maharashtra (new record,); Nepal: Sundarbazar, Lamjung.

Associated plants: Ashoka *Polyalthia longifolia*, common rattlepod *Crotalaria retusa*, flame of the forest *Butea monosperma*, guava *Psidium guajava,* mulberry (*Morus alba*), copper leaf (*Acalypha wilkesiana*), giant

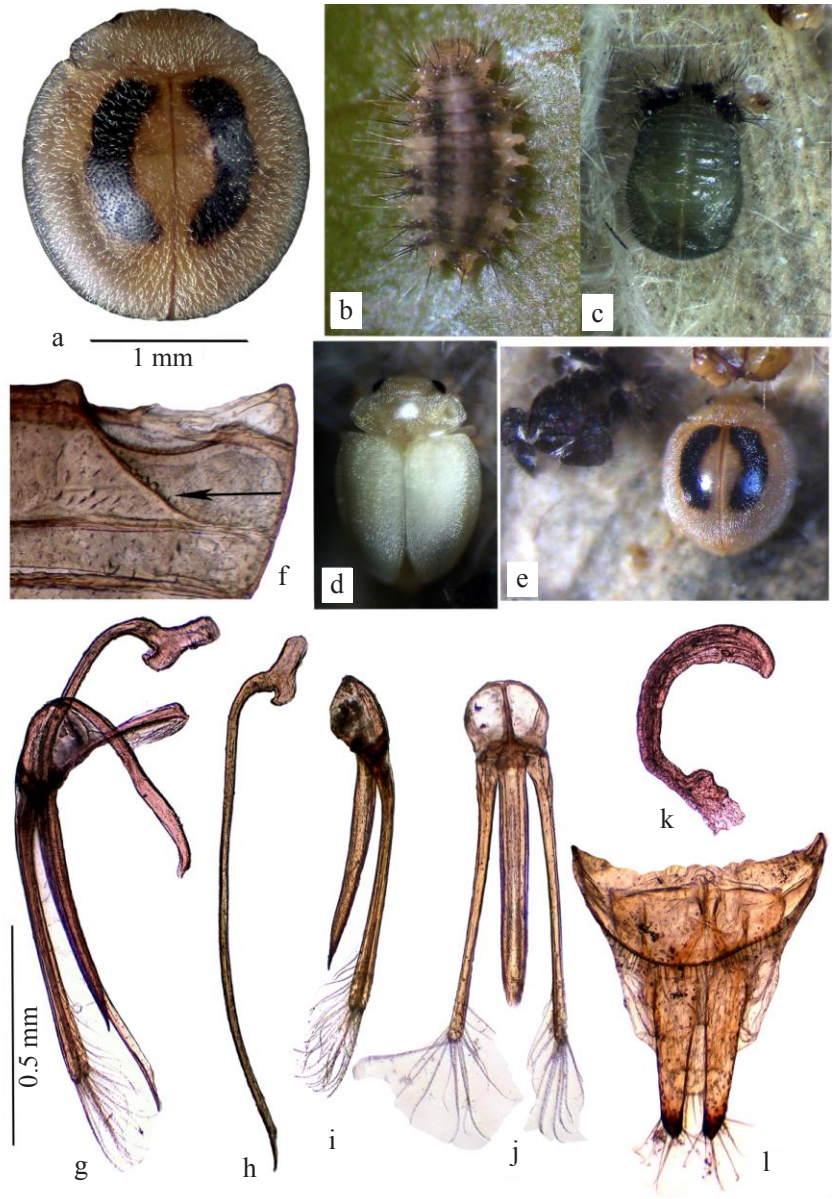


Fig. 1. *Jauravia assamensis*: a, habitus; b, last instar larva; c, 5 day old pupa; d. newly emerged adult; e. matured adult; f, abdomen ventral- arrow indicates incomplete postcoxal line; g-I, male genitalia- h, penis, i,j- tegmen, lateral and ventral view; k,l-female genitalia, k, cornu, l, coxites

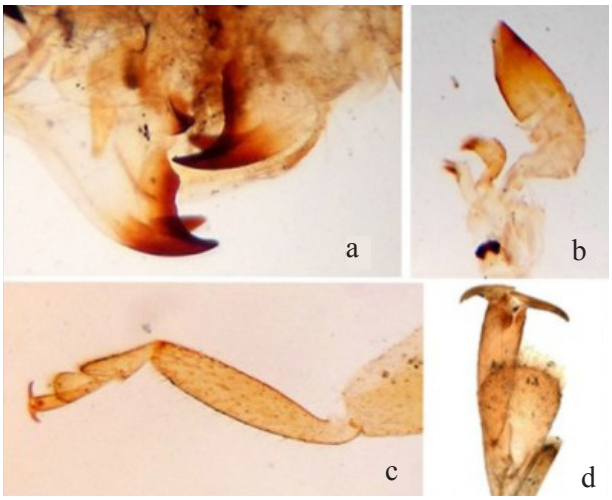


Fig. 2a-d. *J. assamensis*- a, mandibles, b, apically acuminate maxilla, c, pseudotrimerous tarsi, d, simple tarsal claw

milkweed *Calotropis gigantea* and bamboo *Bambusa* spp.

Remarks: The last instar larvae collected from *Polylthia longifolia* were reared providing whitefly infested guava leaf under laboratory conditions which underwent pupation. Adults emerged after five days of a pupal period which were white with yellowish pronotum before sclerotization and then kidney-shaped elytral marks appeared after 3-4 hr later (Fig. 1 b-e). Following Kapur (1961) and Das et al. (2021) the specimens were identified as *J. assamensis;* confirmed with the black spots on the elytra and the male genitalia (penis). The elytral black spots are one-third as long as elytron in *J. kanarensis* (Kapur, 1946), nearly three-fourth as long as elytra in *J. hanifi* (Afroze and Uddin, 1998) as long as elytron and nearly two-thirds as long as elytron in *J. assamensis* (Kapur, 1961) which is similar to elytral spot seen in the specimens studied now. Although *J. hanifi* and *J. assamensis* look a bit similar, these differ in their penis- in *J. hanifi* it is smoothly curved (Afroze and Uddin, 1998), while in *J. assamenis* it is curved at an angle (Kapur, 1961).

The holotype of *J. assamensis* Kapur, 1961 was recorded as feeding on aphids, mites and scale insects from Assam, India; it was also reported from Meghalaya (Khasi Hills) by Chakraborty and Biswas (2002). Chakrabarti et al. (2012) in their studies from Eastern Himalaya and Northeast India mentioned the distribution of *J. assamensis* from Manipur, and observed this to prey upon *Aphis gossypii* Glover and *Myzus persicae* (Sulzer). In the present study, *J. assamensis* was found to be associated with eight plants viz., *Polyalthia longifolia*, *Crotalaria retusa*, *Butea monosperma*, *Psidium guajava, Morus alba*, *Acalypha wilkesiana, Calotropis gigantea* and *Bambusa* spp. The present study documents *J. assamensis* for the first time from Western Ghats and thus, peninsular India showing its range extension.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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DIVERSITY OF LEAFHOPPERS ON FRUIT CROPS AND WEEDS IN NORTH KASHMIR

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ABSTRACT

This study analyses the diversity of leafhopper fauna at six locations (Wadura, Arampora, Dangiwacha, Imberzalwari from District Baramulla and Chogul and Chetkak from District Kupwara) occurring on various fruit crops and weeds. Diversity indices such as species diversity index, evenness index, richness index and abundance were computed. These reveal that six species infest these, of which those infesting fruit trees include *Empoasca decipiens, Empoasca fabae, Agallia* spp.*, Aconeurella erebrus* and *Exitianus indicus*; and *Stirellus* spp., *Maiestas dorsalis, Macrosteles quadrilineatus, Exitianus indicus* and one plant hopper species viz., *Laodelphax striatellus* infest weeds. Maximum abundance was observed to be of *A. decedens* on fruit trees and *Stirellus* spp. on the weed flora.

Keywords: Kashmir, fruit crops, weeds, leafhoppers, planthoppers, diversity indices, abundance, species diversity, richness,

The present study on the fauna of leafhoppers and planthoppers from Kashmir was done to analyse their diversity. These damage crops by either feeding on plant sap or indirectly serving as vectors for plant pathogens. The Cicadellidae (leafhopper) family causes significant harm to crops (Nielson, 1968). Many species feed on herbaceous or woody dicotyledonous plants, while about 1/3 of the tribes specialize on grass and sedge hosts (Zahniser and Dietrich, 2013). Leafhoppers (Cicadellidae) comprise the largest family of Hemiptera with 24,000 described species in over 2600 genera (Dmitriev, 2021). Planthoppers feed on plant sap and damage the plant tissue by ovipositing that lead to wilting of plants (Reissig et al., 1986). Planthoppers can be differentiated from other 'hoppers' by their Y-shaped anal veins in the forewing, and the thick three-segmented antennae (Bay, 2021). This study focuses on the diversity of these hoppers on fruit trees and weeds in some locations of North Kashmir

MATERIALS AND METHODS

The present study was carried by surveying different fruit crops (apple, grapes and walnut) and weeds (white clover, Bermuda grass and wild mint) at different locations viz. a) Wadura; b) Imberzalwari; c) Dangiwacha d) Arampora e) Chogal and f) Chetkak. Hoppers were collected using sweep nets, light traps and sticky traps, killed in ethyl acetate, and sent to IARI, New Delhi for identification. Species diversity for each crop and location was worked out adding up the total number of species found in each community. Different parameters were used to find diversity such as: a) Relative Abundance: = ni/ N x 100 where, ni = Number of Individuals of a single species and N = Total number of individuals of all species b) Species Diversity Index (H): $-\sum_{i=1}^{S} pi\ lnpi$ where H = Shannon- Wiener Biodiversity Index, pi = Relative abundance of each species. ln pi = Natural log of pi. and S = Total number of species. c) Species Richness Index (Ma) = S-1/ 1n N (Pielou, 1975) Where, S = Total number of species collected, N = Total number of individuals in all the species d) Species evenness index: = H/ 1n S where, H = Shannon-Wiener biodiversity Index and S = Total number of species in the community.

RESULTS AND DISCUSSION

The data on the fauna of hoppers on fruit trees and weeds in North Kashmir revealed six species- on fruit trees these include *Assymetrasca decedens, Empoasca decipiens, Empoasca fabae, Agallia* spp.*, Aconeurella erebrus* and *Exitianus indicus;* while on weeds these were *Stirellus* spp., *Maiestas dorsalis, Macrosteles quadrilineatus, Laodelphax striatellus, Exitianus indicus* and *Erythria* spp. (Fig. 1-11). Apple was infested by many species, of which the most abundant was *A. decedens* on apple, grapes and walnut. Of the ones on weeds viz., white clover, Bermuda grass and wild mint, the most abundant species was *Stirellus* spp. (Table 1).

Table 1. Diversity indices and relative abundance of hoppers on fruit crops and weeds in North Kashmir

Crop	Location	MRA						N	S	H	J	Ma
		Assymetrasca decedens	*Empoasca decepiens*	*Empoasca fabae*	*Agallia* spp.	*Aconurella erebrus*	*Exitianus indicus*					
Apple	Wadura	23.80	19.04	19.04	14.28	14.28	9.58	21	6	1.753	0.978	5.671
	Imberzalwari	36.36	27.27	18.18	18.18	0	0	11	4	1.342	0.968	3.582
	Arampora	42.85	28.57	14.28	14.28	0	0	7	4	1.277	0.921	3.486
	Dangiwacha	21.21	18.18	21.21	15.15	15.15	9.09	33	6	1.757	0.980	5.714
	Chogul	23.07	19.23	23.07	15.38	15.38	3.84	26	6	1.695	0.946	5.693
	Chetkak	26.66	20	20	13.3	13.3	6.66	15	6	1.714	0.956	5.630
	Mean	28.99	22.05	19.30	15.09	9.69	4.86	18.83	5.33	1.589	0.958	4.963
Grapes	Wadura	36.36	27.27	18.18	18.18	0	0	11	4	1.342	0.968	3.582
	Imberzalwari	37.5	25	25	12.5	0	0	8	4	1.320	0.952	3.519
	Arampora	60	20	20	0	0	0	5	6	0.950	0.864	2.378
	Dangiwacha	31.57	26.31	21.05	21.05	0	0	19	6	1.371	0.989	3.660
	Chogul	33.33	26.66	20	20	0	0	15	6	1.362	0.982	3.630
	Chetkak	37.5	37.5	12.5	12.5	0	0	8	4	1.320	0.952	3.519
	Mean	39.38	27.12	19.46	14.04	0	0	11	3.83	1.267	0.943	3.381
Walnut	Wadura	38.88	33.33	27.77	0	0	0	36	3	1.089	0.991	2.720
	Imberzalwari	40	33.33	26.66	0	0	0	30	3	1.085	0.987	2.705
	Arampora	35.48	32.25	29.03	0	0	0	31	3	1.085	0.987	2.705
	Dangiwacha	37.20	32.55	30.23	0	0	0	43	3	1.094	0.996	2.734
	Chogul	38.46	33.33	28.20	0	0	0	39	3	1.090	0.992	2.727
	Chetkak	37.93	34.40	27.58	0	0	0	29	3	1.090	0.992	2.703
	Mean	37.99	33.19	28.24	-	-	-	34.67	-	1.090	0.992	2.716
		Stirellus spp.	*Maiestas dorsalis*	*Macrosteles quadrilineatus*	*Laodelphax striatellus*	*Exitianus indicus*	*Erythria* spp.					
White clover	Wadura	50.68	20.54	13.69	10.95	4.10	0	73	5	1.753	0.978	5.671
	Imberzalwari	59.32	16.94	13.55	10.16	0	0	59	4	1.342	0.968	3.582
	Arampora	62.26	15.09	13.20	9.43	0	0	53	4	1.277	0.921	3.486
	Dangiwacha	45.97	20.68	13.79	13.79	5.74	0	87	5	1.757	0.980	5.714
	Chogul	48.10	20.25	13.92	12.65	5.06	0	79	5	1.695	0.946	5.693
	Chetkak	51.51	21.21	13.63	10.60	3.03	0	66	5	1.714	0.956	5.630
	Mean	52.97	19.12	13.63	11.26	2.99	0	69.50	4.66	1.589	0.958	4.963
Bermuda grass	Wadura	61.40	15.78	12.28	0	5.26	5.26	57	4	1.342	0.968	3.582
	Imberzalwari	62.5	15	12.5	0	10	0	40	4	1.320	0.952	3.519
	Arampora	68.75	12.5	12.5	0	6.25	0	32	4	0.950	0.864	2.378
	Dangiwacha	56.92	18.46	13.84	0	7.69	3.07	65	4	1.371	0.989	3.660
	Chogul	60	16.66	13.33	0	5	5	60	4	1.362	0.982	3.630
	Chetkak	66.66	14.58	10.41	0	6.25	2.08	48	4	1.320	0.952	3.519
	Mean	62.71	15.49	12.47	0	6.74	2.56	50.33	4	1.267	0.943	3.381
Wild mint	Wadura	40.54	32.43	0	27.02	0	0	37	3	1.089	0.991	2.720
	Imberzalwari	39.39	33.33	0	27.27	0	0	33	3	1.085	0.987	2.705
	Arampora	38.09	33.33	0	28.57	0	0	21	3	1.085	0.987	2.705
	Dangiwacha	39.53	32.55	0	27.90	0	0	43	3	1.094	0.996	2.734
	Chogul	40	32.5	0	27.5	0	0	40	3	1.090	0.992	2.727
	Chetkak	44.82	31.03	0	24.13	0	0	29	3	1.090	0.992	2.703
	Mean	40.39	32.95	0	27.06	0	0	33.83	3	1.090	0.992	2.716

N = Total no. of individuals of identified species observed/ five sweep nets; MRA: Mean Relative Abundance; S = Total number of identified species; H = Species diversity index; J =Species evenness index; Ma= Species richness index.



Fig. 1. *Empoasca decepiens*; 2. *Empoasca fabae*; 3. *Agallia* spp.; 4. *Aconeurella erebrus*; 5. *Exitianus indicus*; 6. *Stirellus* spp.; 7. *Maiestas dorsalis*; 8. *Macrosteles quadrilineatus*; 9. *Laodelphax striatellus*; 10. *Assymetrasca decedens*; 11. *Erythria* spp.

Diversity indices given in Table 1, reveal that among fruit trees, the value of species diversity index for was maximum of 1.589 on apple and 1.090 on walnut, and of the weeds it was maximum of 1.257 on white clover. Species composition and abundance is usually affected by a combination of geographical and environmental factors including vegetation, topography, altitude, climate, habitat and human influence (Wasowska, 2004). Moreover, altitude also plays an important role in the distribution of plant and animal species with large numbers being recorded at lower altitudes which might be related to temperature which decreases with altitude (Alexander and Hillard, 1969). Hopkin's (1919) bioclimatic law states that there is a four day delay in the hatching of insect eggs for every 400 feet increase in altitude which could affect the population density and abundance of species along altitudinal gradient. A greater species diversity also occurs at lower altitudes due to longer seasons (Alexander and Hillard, 1969).

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ODONATA DIVERSITY IN THE GANGETIC PLAIN OF WEST BENGAL

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ABSTRACT

The diversity and abundance of the Odonata documented from the Gangetic plain of West Bengal covering two types of habitats revealed its primarily aquatic nature (Oxbow Lake, Purbasthali). The other sites were predominantly grasslands, with smaller water bodies around (RRS, Chinsurah and DVC canal, Baidyabati). A total of 40 species were documented belonging to 31 genera and 5 families, with dominant families under the suborders Anisoptera and Zygoptera being Libellulidae and Coenagrionidae, respectively. Shannon-Wiener diversity index indicates that Oxbow Lake, Purbasthali has the most diverse fauna, with a higher relative abundance for Anisoptera compared to the Zygoptera.

Key words: Anisoptera, Zygoptera, diversity indices, genera, families, relative abundance, aquatic, dominance status, Libellulidae, Coenagrionidae, Shannon- Wiener index

The order Odonata, comprising of dragonflies and damselflies, is one of the fascinating insect groups. These are important components of freshwater ecosystems as well as good indicators of ecosystem health (Clark and Samways, 1996). Till date, globally 5,680 species of odonates are known, of which 588 species are found in India. Of these 251 species are endemic (Kalkman et al., 2020). Odonates occur close to fresh water habitats viz. rivers, streams, lakes, pools and rice fields (Tiple et al., 2012), and form one of the important invertebrate predators (Sharma et al., 2007). The Odonata studies in West Bengal were initiated by Selys (1891) who described 22 species from Kolkata. Later, Mitra (2002) recorded 65 species from Kolkata and its adjoining area, mainly Howrah District. Srivastava and Sinha (1993) reported 178 species from West Bengal. Later on several studies were carried out neighboring this locality by Dwari and Mondal **(**2017), Dawn P (2014; 2021), Pahari et al. (2019), Mandal and Aditya (Bandopadhyay) (2017), Payra and Tiple (2019) etc. Research on the Odonata diversity in the Gangetic plain in West Bengal is limited. The present study was undertaken in this locality to document their diversity and relative abundance.

MATERIALS AND METHODS

This study was conducted in the Rice Research Station (RRS), Chinsurah, (Site 1- 22˚52'N, 88˚24'E, 8.62 masl) (Bhowmick et al., 2014). DVC Canal, Baidyabati (Site 2) is located in between Bhadreswar and Badyabati railway station (22˚47'N, 88˚19'E). This area has a canal connected to river Ganges and agricultural fields mainly paddy fields. Oxbow Lake, Purbasthali, Burdwan (Site 3) lies on the Tropic of Cancer in the quaint town of Purbasthali, in Burdwan, West Bengal (23˚27'N, 88˚20'E, 16 masl). The study was conducted from March 2018 to March 2021. All the sites were visited twice monthly from premonsoon to post monsoon, from March to November. Common species were identified and photographed in the field; some doubtful specimens were captured with butterfly net, and identification done following the keys of Fraser (1933; 1934; 1936), Subramanian (2009), Mitra (2002) and Nair (2011). Field photographs were taken using Canon 80D camera with 100 mm prime macro lens (Canon). The odonates were categorized into four groups (very common, common, rare and very rare) on the basis of their frequency of sightings in all the study areas.

RESULTS AND DISCUSSION

A total of 40 species of Odonata representing 31 genera from 5 families were observed (Table 1; Figs. 2-25. Among the dragonflies (suborder Anisoptera), the most diverse and abundant family was Libellulidae represented by 25 species (62.5%), followed by the Aeshnidae represented by two species (5%). Such a preponderance of Libellulidae over other families was also well established in different regions of India-Arulprakash and Gunathilagaraj (2010), Tiple et al. (2012), Pahari et al. (2019) and Nayak and Roy (2016). The third family of the Anisoptera is the Gomphidae, represented by a solitary species (2.5%). Among the

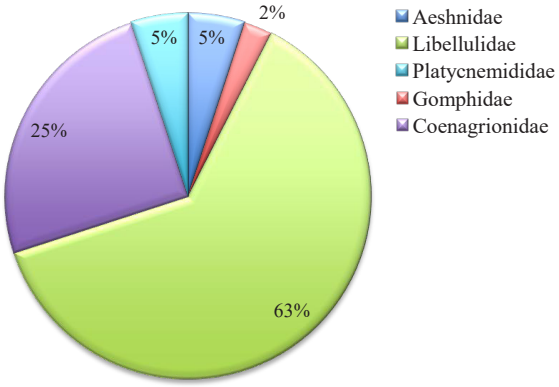


Fig. 1a. Family wise distribution

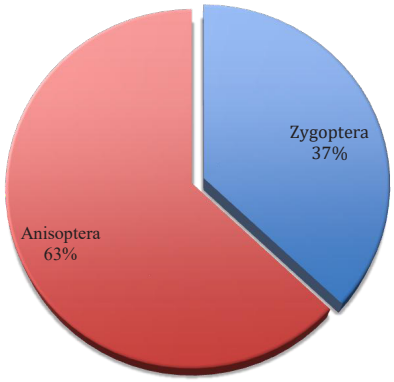
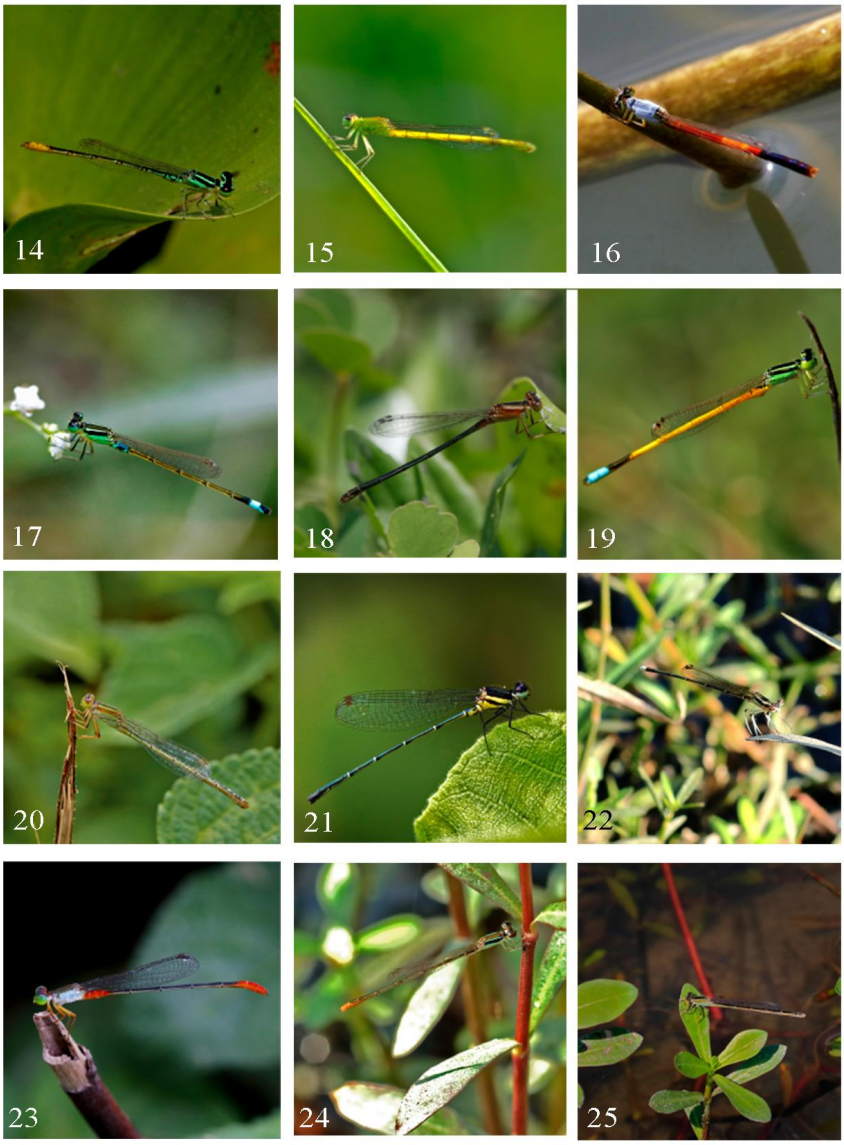


Fig. 1b. Relative abundance of Anisoptera and Zygoptera



Figs. 2-13. Some representative Anisopteran specimens found in this study; 2. *Acisoma panorpoides,* male; 3. *Acisoma panorpoides,* female; 4. *Pantala tlavescens,* male; 5. *Brachythemis contaminata,* male; 6. *Brachythemis contaminata,* female; 7. *Pantala tlavescens,* female; 8. *Crocothemis seiVilia,* male; 9. *Crocothemis seiVilia,* female; 10. *Diplacodes trivialis,* male; 11. *Macrodiplax cora,* male; 12. *Macrodiplax cora,* female; and 13. *Zyxomma petiolatnm,* female



Figs. 14-25. Some representative Zygopteran specimens found in this study; 14. *Agriocnemis kalinga,* male; 15. *Agriocnemis kalinga,* female; 16. *Ischnura nursei,* ma1e; 17. *Isclrnura senegalensis,* ma1e; 18. *Ischnura senegalensis,*female; 19. *Ischnura rubilio,* male; 20. *Ischnura rubilio,* female; 21. *Onychargia atrocyana,* male; 22. *Copera ciliata,* ma1e; 23. *Ceriagrion cerinorubellum,* male; 24. *Agriocnemis pygmaea,* male; and *Agriocnemis pygmaea,* female.

damselflies (suborder Zygoptera), Coenagrionidae was the dominant with 10 species (25%), followed by Platycnemididae (2 species- 5%) (Fig. 1a). It was found that the damselfly *Pseudagrion coromandelianum* was the most abundant species, followed by the dragonflies *Pantala flavescens*, *Crocothemis servilia*, and *Orthetrum sabina*. The relative abundance of the Anisoptera was fairly high (63%) than Zygoptera (37%) (Fig. 1b). This may be due to their larger body size and wider distributional pattern (Norma-Rashid, 2001). Of all the species, 32.5% were very common, 22.5% each fell into common, rare and very rare category. Most of the odonates documented now belonged to the least concern category according to the IUCN red data list. In 75% of the cases both the male and female specimens of the same species were photographed, for the rest either sex was photographed. One species that is *Hydrobasileus croseus,* Brauer was recorded for the first time from this region (Shee et al., 2020). Of the three-study areas, the Ox-bow Lake in Purbasthali seems to be more stable as a habitat for Odonata with lesser threat, indicated by the highest Shannon-Wiener diversity index (Table 2). When relative abundance was calculated it was found that of all the species 8 belonged

Table 1. List of Odonata documented in study areas

S. No.	Scientific Name	Site 1	Site 2	Site 3	RA	Status
Family: Gomphidae						
1.	*Ictinogomphus rapax* Rambur	+++	+++	++	0.77	SR
Family: Aeshnidae						
2.	*Anax guttatus* Burmeister	-	-	+	0.09	SR
3.	*Anaciaeschna jaspidea* Burmeister	+	-	+	0.06	SR
Family: Libellulidae						
4.	*Acisoma panorpoides* Rambur	++++	++++	+++	2.65	R
5.	*Crocothemis sevilia* Drury	++++	++++	++++	11.14	D
6.	*Diplacodes trivialis* Rambur	++++	++++	++++	1.67	R
7.	*Diplacodes nebulosa* Fabricius	++	+	++	0.15	SR
8.	*Macrodiplax cora* Brauer	++	++	+	3.32	SD
9.	*Brachudiplax chalybdea* Brauer	+++	+++	++	2.32	R
10.	*Brachydiplax sobrina* Rambur	+++	+++	++	1.35	R
11.	*Brachythemis contaminata* Fabricius	++++	++++	++++	8.59	SD
12.	*Bradinopyga geminate* Rambur	+	+	-	0.07	SR
13.	*Neurothemis fulvia* Drury	++	+	-	0.36	SR
14.	*Neurothemis tullia* (Drury)	+++	+++	++	1.43	R
15.	*Orthetrum sabina* Drury	++++	++++	+++	5.38	SD
16.	*Orthetrum triangulare* Selys	+	-	-	0.02	SR
17.	*Pantala flavescens* Fabricius	++++	++++	+++	13.10	D
18.	*Potamarcha congener* Rambur	++	++	+	0.83	SR
19	*Rhodothemis rufa* Rambur	++	++	+	0.64	SR
20.	*Rhyothemis variegate* Linnaeus	+++	++++	++++	4.06	SD
21.	*Tholymis tillarga* Fabricius	++	++	++	0.94	SR
22.	*Trithemis pallidinervis* Kirby	+++	+++	++	2.69	R
23.	*Urothemis signata* Rambur	+	+++	-	0.94	SR
24.	*Cratilla lineata* (Brauer)	+	-	-	0.01	SR
25.	*Zyxomma petiolatum* Rambur	+	+	+	0.17	SR
26.	*Orthetrum pruinosum* Burmeister	+	-	-	0.08	SR
27.	*Hydrobasileus croseus* (Brauer)	+	-	-	0.03	SR
28.	*Tramea limbata* Desjardins	+	-	+	0.11	SR
Family: Coenagrionidae						
29.	*Ceriagrion cerinorubellum* Brauer	++	+++	+++	0.95	SR
30.	*Agriocnemis kalinga* Nair & Subramanian	+	+	++	0.60	SR
31.	*Agriocnemis pygmaea* Rambur	++++	++++	++++	6.54	SD
32.	*Ischnura nursei* Morton	-	-	++	0.26	SR
33.	*Ischnura rubilio* Selys	+	+	+++	0.54	SR
34.	*Ischnura senegalensis* Rambur	+++	+++	++++	3.51	SD
35.	*Paracercion malayanum* Selys	-	+	++++	2.53	R
36.	*Ceriagrion coromandelianum* Fabricius	++++	++++	++++	14.88	D
37.	*Pseudogrion microcephalum* Rambur	+	+	++++	4.34	SD
38.	*Pseudogrion rubriceps* Selys	+	++	++++	1.67	R
Family: Platycnemididae						
39.	*Copera ciliate* Selys	+	-	++	0.26	SR
40.	*Onychargia atrocyana* Selys	+	+++	++	0.96	SR

*Abundance of different species in different locations are indicated with the help of + sign. Very rare species is given '+', for rare '++' is used, for common species '+++' is used and '++++' is used for very common species. The absence of any specimen from any site is given '-' symbol.

[Relative abundance (RA) <1 = subrecedent (SR); 1–3.1 = recedent (R); 3.2–10 = subdominant (SD); 10.1–31.6 = dominant (D); >31.7 = eudominant (ED)]. (Engelmann 1973).

Table 2. Species Diversity, Evenness and Dominance indices in the Study area

Location	Indices			
	Shannon Wiener	Margalef's species richness	Pielou evenness (J')	Simpson's species dominance (D)
Site 1	2.73	4.466	0.756	0.094
Site 2	2.772	3.634	0.807	0.089
Site 3	2.806	3.913	0.802	0.085

The Pielou Evenness index or J' value ranges from 0 to 1. Higher the diversity greater is the value. For Simpsons dominant index (D), the value zero means infinite diversity and the value 1 means no diversity at all. Simpson's index is usually expressed as its inverse (1/ *D*) where greater the number more the diversity.

to recedent, 7 belonged to subdominant, 3 were from dominant category and rest 22 was subrecedent types (Table 1).

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CONFLICT OF INTEREST

No conflict of interest.

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SUITABILITY OF BLACK SOLDIER FLY LARVAE AS HOST FOR ENTOMOPATHOGENIC NEMATODES

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ABSTRACT

Black soldier fly *Hermetia illucens* (L.) larvae can serve many uses, of which potential exists for its use as a host for mass production of entomopathogenic nematodes. This study evaluates its larval instars for their suitability for rearing of *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser). The results revealed that 4th instar larvae is economical and effective, and this can be used at farm level. Thus, it can be popularized for mass production of entomopathogenic nematodes. The nematodes/ cadaver was found to be half as that from *Galleria*, and hence two 4th instar cadavers can be recommended in place of a single *Galleria* cadaver. The results also showed that *H. illucens* is unsuitable for mass multiplication of *S. carpocapsae*.

Key words: *Hermetia illucens*, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Galleria mellonella*, insect rearing, mass production, larval instars, cadavers

Black soldier fly *Hermetia illucens* (L.) (Stratiomyidae: Diptera) is a beneficial insect as it can be used for waste management with minimum carbon footprint, besides its other uses. It can be easily reared in any organic waste material and its biology had been studied by Sharanabasappa et al. (2019), and with biological parameters compared on different food wastes (Srikanth and Deshmukh, 2021). The present study assessed the use of *H. illucens* larva as host for the rearing of entomopathogenic nematodes. These nematodes (EPN) are being advocated as the safest biopesticides. Entomopathogenic nematodes of genera *Steinernema* and *Heterorhabditis* are efficient biocontrol agents (Koppenhofer, 2000). Nematode infected cadavers of greater wax moth (*Galleria mellonella* L.) larvae are the popular ones for the rearing of these nematodes, but their rearing and maintenance requires sufficient expertise and care. This study evaluates the suitability of *H. illucens* larvae as hosts for cost effective mass multiplication of some entomopathogenic nematodes.

MATERIALS AND METHODS

The experiment was conducted at the Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala (N8^{0}25'37.13952", E76^{0}59'12.50736", 29 masl) under laboratory condition during 2020-21. Egg masses of *H. illucens* were collected in cardboard honeycombs from a compost bin set up for attracting the naturally occurring females for oviposition (Ewusie et al., 2019). These were transferred to plastic pots (17 and 25 cm dia, 30cm high) containing vegetable and fruit waste. Suitable larval instars were collected from these and simultaneously reared final instar larvae of *G. mellonella* on standard semisynthetic diet were used as check. Entomopathogenic nematodes viz., *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser), obtained from the Banana Research Station, Kannara and ICAR-NBAIR Bengaluru, respectively were used. Nematodes were maintained in the laboratory (28±2^{0}C, 60-70%RH) as aqueous suspension as well as infected cadavers. There were 10 treatments (T1: 5th instar BSFL+ *H. bacteriophora*, T2: 4th instar BSFL +*H. bacteriophora*, T3: 3rd instar BSFL + *H. bacteriophora*, T4: *Galleria* larva+ *H. bacteriophora*, T5: 5th instar BSFL+ *S. carpocapsae*, T6: 4th instar BSFL +*S. carpocapsae*, T7: 3rd instar BSFL + *S. carpocapsae*, T8: *Galleria* larva+ *S. carpocapsae*, T9: 6th instar BSFL + *H. bacteriophora* and T10: 6th instar BSFL + *S. carpocapsae*) replicated four times under completely randomized design. One

replication consisted of 10 uniform sized larvae of respective instars in a 90 mm petriplate lined with Whatman number 1 filter paper disc. The larvae were exposed to 100 infective juveniles (IJs) of respective nematodes per larva (Koppenhofer and Kaya 1998).

For this purpose, freshly emerged IJs were collected from Whites trap (White, 1927) in double distilled water and diluted to obtain the required number of IJs; the solution was applied to filter paper disc in petri plate. A waiting period of 10 min was given to ensure uniform distribution of IJs and the uniform soaking of filter paper, larvae were introduced and closed. The petriplates were incubated at laboratory conditions at room temperature. Observations were taken at an interval of 24 hr on mortality. Absolute control was set using double distilled water with above mentioned insects and was also replicated four times. Half of the cadavers formed in various treatments were kept in petri plates in laboratory conditions under room temperature and observed daily for colour change, intactness of cadaver and natural emergence of nematodes. Other half of the cadavers were kept in White trap and number of IJs emerged were observed and counted under stereo zoom microscope on 24 hr basis. Viability of IJs from BSF larvae were found out by infecting them against *Galleria* larvae. The data obtained were subjected to statistical analysis using the WASP-Web Agri Stat Package 2.0 programme developed by ICAR- Central Coastal Agricultural Research Institute, Goa.

RESULTS AND DISCUSSION

The results revealed that treatments involving only double distilled water did not cause mortality (these not shown in tables); all larvae were alive in the treatments T5: 5th instar BSF + *S. carpocapsae*; T9: 6th instar BSF + *H. bacteriophora* and T10: 6th instar BSF + *S. carpocapsae*; and larvae in these successfully completed their lifecycle. Mortality of larvae when observed daily for 10 days, it was seen that after 24 hr, mortality was there only in *Galleria* larvae (Table 1). The EPN, *H. bacteriophora* was able to bring about mortality among *H. illucens* larval instars from second day; and all larvae in T4: *Galleria* larva+ *H. bacteriophora* also died on 2 DAI. On the third day, *S carpocapsae* could bring about 100% mortality of *Galleria* larvae (T8), whereas 100% mortality was observed in T3 (3rdinstar BSF + *H. bacteriophora*) after 5 days. *Galleria mellonella*, the universal host of entomopathogenic nematodes

Table 1. Mortality of BSF larvae and *Galleria* larvae treated with entomopathogenic nematodes

Treat-	Mortality at different days interval (%)										Total
ments	1	2	3	4	5	6	7	8	9	10	
	0	2.5	2.5	5	5	5	5	5	5	5	5
T1	(0.91)c	(5.29)e	(5.29)d	(9.67)de	(9.67)de	(9.67)de	(9.67)d	(9.67)d	(9.67)d	(9.67)d	(9.67)e
	0	37.5	75	80	85	85	85	85	85	85	87.5
T2	(0.91)c	(37.51)d	(61.23)b	(64.18)b	(67.50)b	(67.50)b	(67.50)b	(67.50)b	(67.50)b	(67.50)b	(69.53)b
	0	82.5	82.5	97.5	100	100	100	100	100	100	100
T3	(0.91)c	(65.47)c	(65.50)b	(84.71)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a
	85	100	100	100	100	100	100	100	100	100	100
T4	(67.87)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a
	0	0	0	0	0	0	0	0	0	0	0
T5	(0.91)c	(0.91)e	(0.91)d	(0.91)e	(0.91)e	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)f
	0	0	0	7.5	17.5	17.5	40	45	45	45	35
T6	(0.91)c	(0.91)e	(0.91)d	(11.70)d	(18.34)d	(18.34)d	(38.95)c	(42.12)c	(42.12)c	(42.12)c	(36.22)d
	0	2.5	7.5	32.5	42.5	57.5	67.5	75	80	82.5	77.5
T7	(0.91)c	(5.29)e	(14.05)c	(34.50)c	(40.39)c	(50.14)c	(59.00)b	(63.52)b	(66.53)b	(68.19)b	(61.77)c
	75	92.5	100	100	100	100	100	100	100	100	100
T8	(60.64)b	(78.30)b	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a
	0	0	0	0	0	0	0	0	0	0	0
T9	(0.91)c	(0.91)e	(0.91)d	(0.91)e	(0.91)e	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)f
	0	0	0	0	0	0	0	0	0	0	0
T10	(0.91)c	(0.91)e	(0.91)d	(0.91)e	(0.91)e	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)f
CV	27	22.15	17.67	17.91	21.54	22.1	19.16	16.45	14.95	14.37	8.43
CD (0.05)	5.29	9.10	8.37	9.98	12.62	13.27	12.31	10.75	9.84	9.49	5.44
SEM	3.36	9.93	8.39	11.93	19.10	21.10	18.17	13.87	11.61	10.81	1.90

T1: 5th instar BSF+ *H. bacteriophora*, T2: 4th instar BSF +*H. bacteriophora*, T3: 3rdinstar BSF + *H. bacteriophora*, T4: *Galleria* larva+ *H. bacteriophora*, T5: 5th instar BSF + *S. carpocapsae*, T6: 4th instar BSF +*S. carpocapsae*, T7: 3rdinstar BSF + *S. carpocapsae*, T8: *Galleria* larva+ *S. carpocapsae*, T9: 6th instar BSF + *H. bacteriophora* and T10: 6th instar BSF + *S. carpocapsae;* * Values in parentheses after arc sine transformation

proved its superiority. Alonso et al. (2018) found that *S. carpocapsae* activation was much higher to insect homogenates of *G. mellonella* than to *H. illucens*. This is slightly in disagreement with the findings of Toutoris et al. (2017) who observed that fourth instar of *H illucens* was the most susceptible, and third instar was more prone to speedy nematode infections since both nematodes caused mortality within 48 hr. Later instars of *H. illucens* are known for their tough integument due to deposition of calcium carbonate (Johannsen, 1922); their larvae have only two pairs of functional spiracles and the integument is shagreened due to deposition of calcium carbonate (Stehr, 2008).

Comparing the nematodes, *H. bacteriophora* gave quick mortality than *S. carpocapsae;* the former not only utilized natural openings but it got attached to host cuticle and entered with a drill like mechanism and caused changes in host behavior and produced septicemia within 6 hr in *Drosophila* maggots (Dziedziech et al., 2020). The results clearly indicated that mortality decreased with age of the larvae. A similar trend was also observed by Tourtois et al. (2017). Younger larvae of Mexican fruit fly *Anastrepha ludens* (Loew) were found more susceptible to *H. bacteriophora* (Toledo et al., 2005) than older ones; which is in agreement with the present findings. Susceptibility of younger larvae to EPN was also demonstrated by Fuxa et al. (1988) in the case of *S. feltiae* exposure to fall armyworm larvae.

Number of infective juveniles of EPN that emerged from cadavers were a direct indicator of host suitability for mass multiplication. From the *H. bacteriophora* infected *Galleria* cadaver, 4.51 lakhs of IJs emerged, whereas from 4th instar of *H. illucens*, 2.3 lakhs *H. bacteriophora* IJs were seen emerging. Rahoo et al. (2019) also observed >4.5 lakhs *H. bacteriophora* IJs emerging from a single final instar *G. mellonella* cadaver. The present observations in this respect are not in agreement with that of Tourtois et al. (2017) who observed tenfold less IJs of *H. bacteriophora* from *H. illucens* than from *G. mellonella*, even after injuring the larval cuticle. *Hermetia illucens* BSF was observed not at all suitable for *S. carpocapsae* mass production; though 4th instar larva produced 1.1 lakh IJs; the emergence was not uniform in all replications. Increased infection rate and mortality did not lead to an increase in the number of IJs harvested from *H. illucens* exposed to *Steinernema* spp. (Tourtois, 2014). In the case of *H. bacteriophora*, IJs started emerging from *G. mellonella* and 4th instar of *H. illucens* from the 6th day

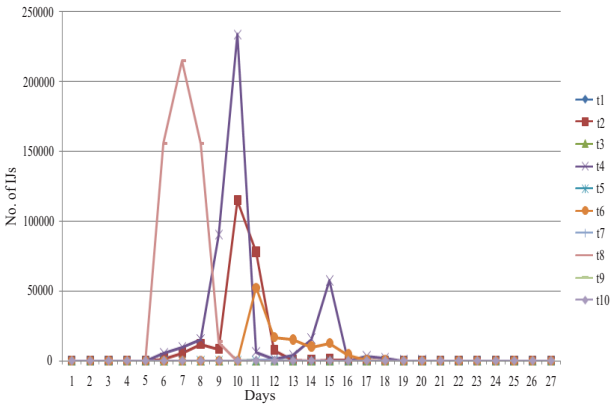
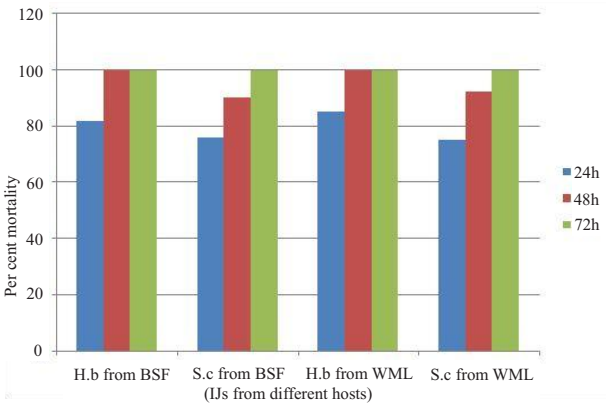


Fig. 1. Daily emergence of infective juveniles from cadavers

but cease to emerge from 27 and 23rd day, respectively (Fig. 1). Peak emergence of *H. bacteriophora* was seen on the 10th day on both of these cadavers. A similar trend was observed by Rahoo et al. (2019) who observed a peak on the 13th day in the case of *H. bacteriophora* from *Galleria* cadavers. For *S. carpocapsae*, infective juveniles emerged from 6th to 16th day, 11th to 21st day and 11th to 13th day in *Galleria*, third and fourth instar *H. illucens* larvae, respectively; though third instar was more susceptible, these produced less nematodes/ insect.

Cadavers formed by *H. bacteriophora* were initially colourless, turning pale yellowish orange within a day, then to brick red and chocolate brown within 4 to 6 days. All cadavers were flaccid initially, but they were seen gaining turgidity slowly within 5 to 7 days. Cadavers formed due to attack of *S. carpocapsae* were initially colourless, flaccid and softer than that of *H. bacteriophora*, turned to pale grey and to dark grey in *Galleria*; while *H. illucens* cadavers also shown variations in colour from colourless to various shades of grey, but the colour did not get intensified. Natural egress of infective juveniles was observed in the case of cadavers of *G. mellonella* (both nematodes) and *H. bacteriophora* infected 4th instar *H. illucens* only. Nematodes were seen emerging from the mouth in the case of *H. illucens* and through all natural openings in *G. mellonella* initially. Infective juveniles of both species of nematodes from *H. illucens* cadavers were found viable since 100% mortality was observed in *Galleria* larvae when inoculated with these IJs emerged from *H. illucens* BSF (Fig. 2). This clearly indicated that EPN emerged from *H. illucens* are as viable and effective as that from *G.mellonella*. Thus, it can be concluded that fourth instar *H. illucens* can be utilized for the mass production of *H. bacteriophora*.



(H.b: *H. bacteriophora*, S.c: *S. carpocapsae*, WML: wax moth larva)

Fig. 2. Mortality of *G. mellonella* larvae by IJs from two hosts

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AUTHOR CONTRIBUTION STATEMENT

MCR, NA and GR conceived research. GR helped in designing the research and provided guidance in carrying out nematological techniques and provided nematode culture. PYPI contributed to statistical analysis. MCR prepared the draft manuscript. All authors reviewed, updated and finalized the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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BIOLOGY OF *ZEUGODACUS TAU* (WALKER) ON BOTTLE GOURD

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ABSTRACT

Biology of *Zeugodacus tau* (Walker) (Diptera: Tephritidae) was studied on bottle gourd under laboratory conditions. It was observed that 4-10 eggs/ female were laid singly or in clusters, embedded vertically or slightly slanting inside the fruits. Preoviposition, oviposition, post-oviposition and incubation periods were observed to be from 10 to 16, 11 to 28, 1 to 4 and 1 to 3 days, respectively. Mean larval period was 1.4, 1.8 and 2.8 days, for 1st, 2nd and 3rd instars, respectively, with total larval duration of 6.0 days. Prepupal and pupal period were 0.9 and 8.2 days, respectively. The mean longevity of male and female was 28.4 and 31.6 days, respectively.

Key words: *Zeugodacus tau*, *Lagenaria siceraria*, biology, morphometrics, developmental stages, instars, larval period, pupal period, oviposition, longevity

The global invasion of Tephritidae (fruit flies) attracts a great deal of attention in the field of quarantine and invasives. The devastating effects that fruit flies inflict to the horticultural crops, and the transboundary nature, have made them as key pests (Enkerlin, 2003). *Zeugodacus tau* (Walker) (Diptera: Tephritidae) is an economically important pest known from almost all parts of the Indian subcontinent. It is a pest on a wide variety of food plants and in certain seasons it causes havoc (Shen et al., 2014). In Asia, approximately 30-40% of losses are caused by *Z. tau* infestation (Jaleel et al. 2018). The female fly punctures the soft and tender fruits with the ovipositor and lays eggs below the exocarp of the fruit. The maggots that hatch from the eggs bore into the fruit and feed on the placenta with secondary infection, resulting in rotting and premature fall of fruits. The knowledge of pest biology has not only academic or scientific importance, but it also points the weak linkage used for development of effective management strategy. Detailed understanding of the biology of the pest like *Z. tau* will be very helpful in devising effective IPM. The present study is on the biology of *Z. tau* on bottle gourd under laboratory conditions.

MATERIALS AND METHODS

The culture of *Z. tau* was initiated with the maggots collected from bottle gourd field and study was done during kharif 2019 in the Division of Entomology (21.4± 7.32°C; 23-94% RH). The infested fruits were kept in 20x 20x 8 cm plastic trays on a 5 cm-thick layer of sieved moist sand to facilitate pupation. The newly emerged adult flies were collected and placed inside the rearing cages (35x 30x 35 cm), provided with glucose solution (10% W/V) and slices of bottle gourd as a source of food and site for oviposition. A compound microscope with slide mounted specimen was used to record the number of eggs present in each bottle gourd slice. The eggs collected were placed in 5 cm dia petridishes with moist filter paper at the bottom to prevent desiccation. Morphometrics of life stages were carried out using calibrated ocular micrometer and vernier caliper. Ten replicates of each stage viz. egg, freshly hatched maggots, full grown maggot, pupae and adult were maintained for linear measurements. In addition to the above parameters colour, shape and period for each lifestage were observed. Observations on preoviposition, oviposition and postoviposition periods, fecundity, incubation period, egg hatchability, larval, prepupal, and pupal periods, adult longevity and sex ratio were also observed.

RESULTS AND DISCUSSION

Eggs of *Z. tau* were shiny white, elliptical, smooth, slightly curved, elongate, tapering anteriorly with slightly protruding micropylar opening while rounded posteriorly and turning darker as hatching approached. Morphometric observations revealed that the egg measured 1.26± 0.15 (length) and 0.24± 0 .04 mm (breadth) (Table 1). Incubation period lasted for

Table 1. Biology and morphometrics of *Z. tau*

Stage	Length (mm)		Breadth (mm)	
	Range	Mean± SD	Range	Mean± SD
Egg	0.96-1.37	1.26± 0.15	0.2-0.34	0.24± 0.04
		Maggot		
1st Instar	1.21-3.72	2.68± 0.98	0.22-0.55	0.42± 0.12
2nd Instar	4.08-7.59	5.65± 1.23	1.04-1.46	1.29± 0.12
3rd Instar	7.98-10.79	9.16± 1.05	1.38-2.82	1.84± 0.49
Prepupa	6.22-7.33	6.74± 0.43	1.94-2.44	2.13± 0.19
Pupa	4.92-5.91	5.48± 0.35	1.86-2.69	2.31± 0.24
		Adult (with expanded wings)		
Male	6.75-8.81	7.76± 0.75	10.31-15.10	13.85± 1.40
Female	8.19-10.9	9.24± 0.78	14.88-17.35	16.55± 0.69

Life stages	*Range	Mean± SD
Incubation period (days)	1-3	2.3±1.35
Maggot Period (days)		
1st instar	0.5-2	1.4± 0.81
2nd instar	1-3	1.8± 0.63
3rd instar	2-3	2.8± 0.67
Total maggot period (days)	3.5-8	6.0± 2.11
Prepupal period (days)	0.5-2	0.9± 0.55
Pupal period (days)	6-10	8.2± 1.31
Mating period (hours)	2-4.5	3.1± 0.97
Preoviposition period (days)	10-16	12.1± 1.2
Oviposition period (days)	11-28	16.3± 6.8
Post-oviposition (days)	1-4	2.4± 0.7
Fecundity	57-92	73.6± 15.0
Hatching %	81-86	83.0± 3.4
Adult longevity (days)		
Male	17-38	28.4± 2.6
Female	23-39	31.6± 1.1
Temperature (°C)	13.4-29.3	21.4± 7.32
Relative humidity (%)	23-94	46.6± 11.52

2.3± 1.35 days. The present results corroborate with the earlier findings of Kabir et al. (1997) who found incubation period in *Z. tau* as 1-3 days; Patel and Patel (1998) and Naik et al. (2017) who observed this as 1-2 days; and Singh et al. (2010) and Vasudha et al. (2019) as 1.30 days, when reared on pumpkin and bitter gourd, respectively. Larvae passed through three instars, are apodous and frugivorous with an elongated body, pointed anteriorly or cephalic and blunt posteriorly. Freshly emerged first instar was translucent, pale white, gut content visible through translucent skin and cephalopharyngeal skeleton weakly sclerotized except for anterior portion of pharyngeal sclerite. Second instar maggot was creamy white in colour, elongate, ellipsoidal in shape, body weakly sclerotized except for anterior portion of pharyngeal sclerite, anterior spiracles well developed and similar to third instar.

The first and second instar maggot measured 2.68± 0.98 and 5.65± 1.23 mm in length and 0.42± 0.12 and 1.29± 0.12 mm in breadth, respectively. The full grown third instar maggot was creamy white with an elongated body, tapering anteriorly except the eighth segment which is truncated, blunt posteriorly and has a tapered head with black oral hooks. It exhibited a peculiar habit of leaping into the air, this hopping behaviour assisted dispersal of the maggots and to find a suitable place for pupation. Length and breadth of full-grown maggot was 9.16± 1.05 and 1.84± 0.49 mm, respectively (Table 1); and duration was 1.4± 0.81, 1.8± 0.63 and 2.8± 0.67 days, respectively. The total maggot period was 6.0± 2.11 days agreeing with those of Patel and Patel (1998), Liu and Lin (2000), Mir et al. (2014), Naik et al. (2017) and Vasudha et al. (2019). Full grown maggots were slightly bent, and stopped feeding, became stationary and measured 6.74± 0.43x 2.13± 0.19 mm, with duration of 0.9± 0.55 days. Pupation occurs in the moist soil or sand, freshly formed pupae were brownish yellow, segmented, barrel shaped and rounded anteriorly; and later the colour changed into

pale brown to brownish grey with eleven segments; it measured 5.48± 0.35 x 2.31± 0.24 mm with duration of 8.2± 1.31 days, these results agree with earlier ones (Singh et al., 2010; Waseem et al., 2012; Laskar, 2013 and Vasudha et al., 2019).

The freshly emerged adult flies were less active, pale yellow with wings attached to the body. After emergence, adults crawled to a sheltered spot nearby until their wings unfold and dry. The flies became brown or ferruginous brown after 2-3 hr. Two round spots on the face, conspicuous black or dark brown markings on lateral and median part of thorax and typical T shaped marking on abdomen. Wings are large with fuscous shadings on the outer margin and costal band largely expanded into a distinct spot at the apex. Adults are moderate in size; female flies are bigger, are easily distinguishable by the presence of a tapering abdomen ending in a pointed ovipositor. These findings confirm with those of Kitthawee and Rungsri (2011), Mir et al. (2014), Sharma et al. (2017), Naik et al. (2017), Leblanc et al. (2019) and Vasudha et al. (2019). Mating was observed in late afternoon hours. A prolonged mating period, which include courtship and copulation ranged from 2-4.5 hr observed now is in conformity with the findings of Singh et al. (2010), Waseem et al. (2012) and Mir et al. (2014) who found prolonged mating period upto 4 hrs. The preoviposition, oviposition and post-oviposition periods varied from 10-16, 11-28 and 1-4 days, respectively. These findings agree with those of Mir et al. (2014), Sharma et al. (2017), Naik et al. (2017) and Thakur et al. (2017). Fecundity was 73.6± 15.0, with eggs laid at intervals of 1-4 days. The hatching of eggs was 83.0± 3.4. These results corroborate with those of Mir et al. (2014) and Thakur et al. (2017). Male and female longevity was 28.4± 2.6 and 31.6± 1.1 days, respectively agreeing with earlier results of Singh et al. (2010), Mir et al. (2014) and Vasudha et al. (2019).

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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INSECT PEST SPECTRUM OF FRENCH BEAN *PHASEOLUS VULGARIS* (POLE TYPE) GROWN UNDER NETHOUSE

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ABSTRACT

Pole bean (*Phaseolus vulgaris* L.) is an annual herb grown vertically on a support. The present study asseses the insect pests infesting this bean along with their natural enemies under nethouse conditions. The study was done at the Hi-tech Horticulture Unit, UAS, Dharwad. The results revealed five species of insects and one mite species infesting pole bean at various phases of crop growth. These include- the serpentine leaf miner (*Liriomyza trifolii*), pod fly (*Melanagromyza obtusa*), seed fly (*Anthomyia* sp.), leafhopper (*Ianagallia bifurcate*), tobacco caterpillar (*Spodoptera litura*), and red spider mite (*Tetranychus urticae*). The natural enemies include- *Brachymeria* sp. (Chalcididae), *Ichneumon* sp. (Ichneumonidae) and *Microplitis* sp. (Braconidae).

Key words: *Phaseolus vulgaris*, nethouse, insects, mites, natural enemies, crop stages, Braconidae, Chalcididae, leaf miner, tobacco caterpillar, leafhopper, seed fly

India is the world's leading producer of pulses and accounts for 26% of world production and 30% of consumption. French bean *Phaseolus vulgaris* L. is a herbaceous plant that belongs to the legume family Fabaceae. Vegetable cultivation under protective structures like the nethouse has become very popular now (Kaur et al., 2004). But, growing vegetable crops under nethouse makes them more susceptible to pests due to warm and humid conditions, and it is necessary to know the pest diversity, their abundance and the associated natural enemies, in these. The major pests of green beans as reported by Mondal et al. (2018) are bean stem fly (*Ophiomyia phaseoli* Tryon), aphids (*Aphis craccivora* Koch), mites (*Tetranychus* sp.), whitefly (*Bemisia tabaci* Gennadius), leaf miner, (*Liriomyza trifolii* Burgess), pod borers like *Helicoverpa armigera* (Hubner) and bean gall weevil (*Alcidodes signatus* Boheman), under open field conditions. Since, pole type French bean is being newly introduced in North Karnataka, the present study assessed its insect pest spectrum along with their natural enemies under net house conditions.

MATERIALS AND METHODS

Insect pests and natural enemies of French bean (pole type) under nethouse was analysed during rabi 2019-20, at the Hi-tech Horticulture Unit, UAS, Dharwad (15.4889 °N,74.9813 ° E). Experiment with variety Indus IZ Polo with a plot size of 500 m^2 and spacing of 30x 45cm was laid out, kept free from chemicals, and there were 10 subplots of 5 m^2 each. To know the pest spectrum, scouting was done by looking at the plants in all its growth stages, from the bottom to top i.e. older leaves to new flush. For calculating the abundance, Gupta et al. (2016) was followed; in case of leaf miner, the number of larvae/ five leaves from five tagged plants, and from each subplot was recorded. The leafhoppers (including nymphs) and mites were counted on three leaves, collected from top, middle and bottom portion of the randomly tagged five plants. For tobacco caterpillar, number of larvae/ m length in five randomly selected spots/ subplot was recorded. Insects and natural enemies prevailing from sowing to harvest were observed at weekly intervals. Collected insects were observed, sorted, and counted under a stereozoom microscope. Standard procedures were followed for collection and preservation of specimens (Gullan and Cranston, 2014).

RESULTS AND DISCUSSION

Results revelaed occurrence of five insect pests and one mite viz. serpentine leafminer *Liriomyza trifolii* Burgess, bean pod fly *Melanagromyza obtusa* Malloch, bean seed fly *Anthomyia* sp., leafhopper *Ianagallia bifurcate* Sawai Singh and Gill, tobacco caterpillar *Spodoptera litura* (F) and red spider mite *Tetranychus urticae* Koch. Allen et al. (1996) and Mehta et al. (2001) reported bean flies, cut worms, serpentine leaf miner,

and spider mites as pests on common beans *Phaseolus vulgaris* L. under field conditions. Similar observations were made by Sood (2010) on beans under the greenhouse. Manjesh et al. (2017) reported incidence of *L. trifolii* and *T. urticae* on yard long beans under shade-nethouse. The activity of *L. trifolii* started at four-leaf stage (two weeks after sowing) and continued throughout the cropping season; peak incidence (6.32 larvae/ 5 leaves) was observed in the pod initiation stage and minimum (2.16 larvae/ 5 leaves) during the early vegetative stage (Table 1). Mondal et al., (2018) observed a peak abundance of leaf miner (4.13 larvae/ plant) on French bean; and Manjy (2019) on cowpea that it was in peak incidence with 10.5 larvae/ leaf. Incidence of *T. urticae* started three weeks after sowing (3.52 mites/ 3 leaves) with peak (10.48 mites/ 3 leaves) during reproductive stage (Table 1); Anuradha (2013) observed peak incidence at 30 days after sowing, under polyhouse on pole bean; Saloni (2018) observed *T. urticae* in nethouse with capsicum; Khatak et al. (2020) observed these on tomato leaves under screen house.

Leafhoppers (*I. bifurcata*) were first noticed at vegetative stage (0.4 to 0.84/ 3 leaves) with peak at reproductive stage (Table 1). Sharma (2008) observed maximum incidence at the late vegetative stage on French bean; mean incidence of *Empoasca fabae* Harris was between 0.41 and 0.73 individuals/ 6 leaves on Indian bean (Chouragade et al., 2018). The infestation of *S. litura* commenced two weeks after sowing, in the early vegetative stage, it was @0.16 larvae/ m with a peak of 0.75 larvae/ m during the flowering stage (Table 1). Manjesh et al. (2017) reported 0.91 larvae/ plant on yard long beans under polyhouse; while with by beet army worm (*S. exigua*) a peak of 1.47 larvae/ plant was during the late vegetative stage on capsicum under shade-net house (Gupta et al., 2016). Earlier findings suggested spider mites and leafhoppers as major sucking pests on French bean (Jakhar and Choudhary, 2013). Saha et al. (2015) observed *L. trifolii* and mites as important pests on beans under protected conditions; and *L. trifolii* was observed maximum on yard long beans under shade-net (Manjesh et al., 2017). The observations on the natural enemies revealed *Brachymeria* sp. (Chalcididae), *Ichneumon* sp. (Ichneumonidae) and *Microplitis* sp. (Braconidae). Xu et al. (2007) found *Opius caricivorae* Fischer, a braconid endoparasitoid on leaf miner. Saha et al. (2015) observed *Dacnusa sibirica* (braconid wasp) on leaf miner. French bean harbours predators like *Coccinella septumpunctata* and *C. dimitida,* syrphid fly, *Chrysoperla carnea* Stephens, rove beetle, and spiders along with parasitoids *Aphidius* sp., *Eretmocerus* sp. and *Campoletes chloridea* Uchida (Mondal et al., 2018). Braconid and eulophid parasitoids were recorded on caterpillar pests under greenhouse (Rathee et al., 2018).

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Table 1. Population abundance of insect pests on pole bean

S. No.	Stage of the crop	Mean incidence			
		Leaf miner/ 5 leaves	Mites/ 3 leaves	Leafhoppers/ 3 leaves	Tobacco caterpillar/ m
1.	Vegetative	2.16	0.00	0.00	0.16
2.	Vegetative	3.16	3.52	0.00	0.38
3.	Vegetative	4.20	4.96	0.00	0.45
4.	Vegetative	4.84	5.80	0.40	0.48
5.	Flowering	5.08	7.52	0.40	0.55
6.	Flowering	5.24	8.84	0.48	0.75
7.	Pod setting	5.88	8.84	0.64	0.60
8.	Pod setting	6.32	9.08	0.68	0.40
9.	Maturity	6.12	10.40	0.84	0.30
10.	Harvesting	6.04	10.48	0.84	0.28
Mean		4.90	7.71	0.61	0.43
S.D±		1.30	3.18	0.32	0.16

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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BIORATIONAL MANAGEMENT OF WHITEFLY *BEMISIA TABACI* (GENNADIUS) IN BRINJAL

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ABSTRACT

Field experiments were conducted during 2019 and 2020 to evaluate the efficacy of six biorational IPM modules against whitefly *Bemisia tabaci* (Gennadius) in brinjal. These included measures like removal of alternate hosts, using of marigold as intercrop/ border row of maize/ yellow sticky traps, release of *Chrysoperla* spp., or use of azadirachtin (1%) and diafenthiuron).The incidence of *B. tabaci* was significantly less in these modules (5.62-7.72 adults/ 3 leaves) compared to untreated control (11.00 adults/ 3 leaves). The maximum fruit yield (437.6 q/ ha)was obtained with module M_1 (removal of alternate hosts + marigold as intercrop and border row of maize + yellow sticky traps + release of *Chrysoperla* spp. + one spray each of azadirachtin (1%) and diafenthiuron 50WP). But, maximum net returns (Rs 25620/ ha) and benefit cost (B: C) ratio (1: 2.2) were obtained with module M_6 (yellow sticky traps+ two sprays of azadirachtin 1%). Thus, it can be concluded that *B. tabaci* can be managed by installing yellow sticky traps @ 100 traps/ ha followed by two need-based sprays of azadirachtin (1 %) @ 1000 ml/ ha.

Key words: *Bemisia tabaci*, brinjal, IPM module, neem, azadirachtin, diafenthiuron, marigold, maize, intercrop, border row, yellow sticky traps, yield, cost benefits

Brinjal (*Solanum melongena* L.) is an important vegetable crop and India ranks second in brinjal production after China (Anonymous, 2018). It is being cultivated throughout the year under irrigated conditions, and in Punjab, it is grown on an area of 5.42 thousand ha with production of 119.7 thousand mt (Anonymous, 2020). Insect pests are the major constraints in its production, of which shoot and fruit borer *Leucinodes orbonalis* Guenee, jassid *Amrasca biguttula biguttula* (Ishida), epilachna beetle (*Epilachna* spp.), whitefly (*Bemisia tabaci* (Gennadius) and aphid (*Aphis gosypii* Glover) are the major pests (Bhadauria et al., 1999).Of these, *B. tabaci* is a pest of global importance, distributed in 162 countries (CABI, 2018) and attack more than 600 plant species (Alemandri et al., 2015). Brinjal is a susceptible host of *B. tabaci* and severe attack may cause considerable damage (Rasdi et al., 2009; Islam and Shunxiang, 2010). Use of insecticides in brinjal results in pesticide residues, and pose serious concerns for human health. So, there is a need of biorational approaches which are effective, economical and less hazardous. This study evaluates some biorational IPM modules against *B. tabaci* in brinjal.

MATERIALS AND METHODS

The field experiments were conducted at the Punjab Agricultural University, Krishi Vigyan Kendra (KVK), Sri Muktsar Sahib (74°30'29''E, 30°26'44''N), Punjab, India during 2019 and 2020. Randomized block design (RBD) was followed with four replications, and in the 1st week of May the healthy seedlings of brinjal (30 days age) were transplanted at 67.5x 45 cm (row x plant spacing) in a plot size of 10x 5 m. A buffer zone of 1.0 m was maintained between two plots. All recommended agronomic practices were followed. Six IPM module formulated on the basis of information available were evaluated along with untreated control. The modules included: M_1 : Removal of alternate hosts + marigold after five rows of brinjal as intercrop and one border row of maize + yellow sticky traps@ 100 ha^{-1} + release of *Chrysoperla* spp larvae@ 10000 ha^{-1} + one spray of azadirachtin (1 %) @ 1000 ml ha^{-1} + one spray of diafenthiuron 50 WP @ 500 g ha^{-1}; M_2 : Removal of alternate hosts + marigold after five rows of brinjal as intercrop + yellow sticky traps @ 100 ha^{-1} + release of *Chrysoperla* spp larvae @ 10000 ha^{-1} + two sprays of azadirachtin (1 %) @ 1000 ml ha^{-1}; M_3: Removal of alternate hosts + yellow sticky traps @ 100 ha^{-1} + release of *Chrysoperla* spp. larvae @ 10000 ha^{-1} + two sprays of azadirachtin (1 %) @ 1000 ml ha^{-1}; M_4 : Removal of alternate hosts + yellow sticky traps @ 100 ha^{-1} + release of *Chrysoperla* spp larvae @ 10000 ha^{-1} + two sprays of PAU homemade neem extract @ 3.0

Table 1. Effect of IPM modules on the incidence of *B. tabaci* on brinjal and their economics (pooled data- 2019, 2020)

Module	Mean no. of whitefly adults/ three leaves																			
	15 DAT	22 DAT	29 DAT	36 DAT	43 DAT	50 DAT	57 DAT	64 DAT	71 DAT	78 DAT	85 DAT	92 DAT	99 DAT	106 DAT	113 DAT	120 DAT	127 DAT	134 DAT	141 DAT	Avg.
M1	3.76	3.92	3.64	4.00	4.26	4.98	2.68	4.33	6.93	9.29	1.01	3.03	4.91	7.47	9.31	11.75	11.18	7.07	3.59	5.62
	(2.18)	(2.22)	(2.15)	(2.23)	(2.29)	(2.44)	(1.91)	(2.31)	(2.81)	(3.20)	(1.41)	(2.00)	(2.43)	(2.91)	(3.20)	(3.57)	(3.49)	(2.84)	(2.14)	(2.51)
M2	3.38	3.95	4.10	4.07	4.27	5.68	2.84	4.23	6.99	11.36	6.36	7.38	9.00	9.68	12.23	13.38	12.14	7.96	4.47	7.10
	(2.09)	(2.22)	(2.25)	(2.25)	(2.29)	(2.58)	(1.96)	(2.29)	(3.83)	(3.51)	(2.71)	(2.89)	(3.16)	(3.27)	(3.63)	(3.79)	(3.62)	(2.99)	(2.34)	(2.80)
M3	3.08	4.29	3.70	4.37	4.36	5.33	2.31	4.45	8.38	10.06	5.76	7.05	8.06	11.11	12.62	13.06	11.91	8.48	5.41	7.02
	(2.01)	(2.30)	(2.16)	(2.31)	(2.31)	(2.51)	(1.81)	(2.33)	(3.06)	(3.33)	(2.59)	(2.84)	(3.01)	(3.48)	(3.69)	(3.75)	(3.59)	(3.07)	(2.53)	(2.78)
M4	2.85	3.98	3.60	5.10	4.17	6.18	3.90	6.05	8.09	12.28	9.99	9.21	10.77	10.08	11.93	13.84	11.99	8.58	5.06	7.72
	(1.96)	(2.22)	(2.14)	(2.47)	(2.27)	(2.67)	(2.21)	(2.65)	(3.01)	(3.63)	(3.31)	(3.18)	(3.42)	(3.33)	(3.59)	(3.85)	(3.60)	(3.09)	(2.46)	(2.89)
M5	3.80	3.89	3.56	4.53	4.55	5.17	4.07	5.49	7.72	10.27	6.17	8.34	9.02	9.48	10.90	12.58	11.17	8.36	4.45	7.00
	(2.19)	(2.21)	(2.13)	(2.35)	(2.35)	(2.48)	(2.23)	(2.53)	(2.95)	(3.35)	(2.67)	(3.05)	(3.16)	(3.23)	(3.45)	(3.68)	(3.49)	(3.06)	(2.33)	(2.78)
M6	3.13	3.54	3.47	3.93	4.32	5.03	6.28	8.59	10.79	9.53	6.97	7.50	7.98	9.88	12.84	6.26	6.77	5.53	3.61	6.62
	(2.03)	(2.12)	(2.11)	(2.22)	(2.30)	(2.45)	(2.70)	(3.10)	(3.43)	(3.20)	(2.82)	(2.92)	(2.98)	(3.29)	(3.72)	(2.69)	(2.79)	(2.55)	(2.14)	(2.71)
Untreated control	3.56	4.78	5.28	6.0	6.35	7.75	9.19	11.60	14.13	15.84	15.38	13.48	13.59	15.43	17.47	17.42	13.94	9.90	7.96	11.00
	(2.13)	(2.40)	(2.50)	(2.65)	(2.71)	(2.96)	(3.19)	(3.55)	(3.88)	(4.10)	(4.05)	(3.80)	(3.81)	(4.05)	(4.30)	(4.29)	(3.86)	(3.30)	(2.99)	(3.40)
CD (p=0.05)	NS	NS	(0.16)	(0.16)	(0.20)	(0.18)	(0.21)	(0.11)	(0.17)	(0.13)	(0.13)	(0.10)	(0.12)	(0.13)	(0.12)	(0.14)	(0.11)	(0.14)	(0.13)	(0.12)

S. No.	Treatment	Yield (q/ ha)	Yield increase over untreated control (q/ ha)	Additional returns due to pest control (Rs/ ha)	*Cost of pest control (Rs/ ha)	Net returns over untreated control (Rs/ ha)	B:C Ratio
1	M_1	437.6[a]	36.6	42090	23318	18773	0.8
2	M_2	429.5[b]	28.5	32775	23823	8953	0.4
3	M_3	425.2[bc]	24.2	27830	23135	4695	0.2
4	M_4	420.8[c]	19.8	22770	19675	3095	0.2
5	M_5	427.3[b]	26.3	30245	12098	18148	1.5
6	M_6	433.2[ab]	32.2	37030	11410	25620	2.2
7	Untreated control	401.0[d]	-	-	-	-	-

Figures in parentheses mean of square root transformations; DAT: Days after transplanting; *Total cost of management module; brinjal price: Rs 11.50/ kg; values with the same letter within a column not significantly different at $p < 0.05$.

litre ha^{-1}, M_5 : Removal of alternate hosts + marigold as intercrop + yellow sticky traps @ 100 ha^{-1}+ two spray of azadirachtin (1 %) @1000 ml ha^{-1}, M_6 : Yellow sticky traps@ 100 ha^{-1}+ two sprays of azadirachtin (1 %) @ 1000 ha^{-1}. Yellow sticky traps, commercial formulation of azadirachtin (1 %) and insecticide diafenthiuron 50 WP were procured from the local market, whereas *Chrysoperla* larvae were obtained from the Biocontrol Laboratory, Department of Entomology, PAU, Ludhiana. PAU homemade neem extract was prepared by using 4 kg neem leaves along with green stem and kernels from terminal portion of shoots were collected from road side trees. The leaves were boiled in 10 ℓ water for 30 min. The solution was filtered through muslin cloth to remove the unwanted material. Observations on adult *B. tabaci* count from three leaves, one each from upper, middle and lower canopy of the plant were recorded at 7 days interval starting from 15 days after transplanting (DAT). Fruits were harvested from each plot separately and the cumulative fruit yield was worked out. Economic analysis was carried out by calculating fruit yield, market value of the produce and cost of variable inputs including cost of predator, sticky traps, insecticides, labour charges etc. The net returns and benefit cost ratio (B: C) was calculated to find out the best module. The yield data was subjected to ANOVA using SPSS version 22 software.

RESULTS AND DISCUSSION

The pooled data revealed that *B. tabaci* incidence was significantly less in the modules (5.62 - 7.72 adults/ three leaves) compared to untreated plots (11.00 adults/three leaves) (Table 1). Integration of biorational IPM components effectively reduced the incidence, and these results corroborate with those of the individual treatments. Lanjar and Sahito (2005) reported that removal of alternate weed hosts from the field significantly reduced the incidence. Durairaj et al. (2007) stated that yellow sticky traps could be used for monitoring, as well as for trapping. Sujayanand et al. (2015) observed that marigold as intercrop and maize as border crop could effectively reduce incidence. The efficacy of diafenthiuron and neem based commercial formulations against *B. tabaci* in brinjal have been documented (Srinivasan and Babu, 2001;Patel et al., 2006). The cumulative yield calculated with pooled data showed significantly less (401.0 q/ha) and maximum (437.6 q/ha) fruit yield in untreated control plots and M_1 module respectively; all the tested modules were found effective giving better fruit yield, but on the basis of economics, maximum net returns (Rs 25620) and B: C ratio (2.2) were obtained in module M_6 (yellow sticky traps @ 100 traps/ ha followed by two sprays of azadirachtin (1%) @ 1000 ml/ ha. Yellow sticky traps and neem-based insecticides could effectively manage *B. tabaci* as observed from earlier studies- Bhonde (2013) against sucking pests in okra; Khaire et al. (2014). Bantewad and Thakare (2017) on cotton; Khuhro et al. (2020) with yellow traps in brinjal; Abbas et al. (2020) with leaf extract of *Azadirachta indica* in cotton under field conditions; Singh and Joshi (2020) with azadirachtin against *Myzus persicae* and *B. tabaci* on capsicum; and Ghongade and Sangha (2021) with azadirachtin in cucumber under greenhouse conditions.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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EVALUATION OF INSECTICIDES AGAINST GALL WEEVIL *ALCIDODES COLLARIS* (PASCOE) ON PIGEONPEA

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ABSTRACT

Pigeonpea is attacked by many insect pests, and of these the gall weevil *Alcidodes collaris* (Pascoe) causes threat to pigeonpea at seedling stage by attacking basal portion of the stem and resulting in the gall formation. Evaluation of some insecticides revealed that less incidence was observed using seed treatment with imidacloprid 600FS followed by chlorantraniliprole 18.5SC spray (1.17 galled plants/ 10 plants) after 20 days of emergence. This also resulted in maximum reduction of galled plants (79.37%). Imidacloprid 600FS seed treatment with drenching of profenophos 50EC at 20 days after the crop emergence was observed to be the next best. Seeds treatment with imidacloprid 600FS followed by chlorantraniliprole 18.5SC spray gave maximum yield of 23.38 q/ ha.

Key words: Pigeonpea, *Alcidodes collaris*, seed treatment, imidacloprid 600FS, chlorantraniliprole 18.5SC, profenophos 50EC, galled plants, yield

Pigeonpea *Cajanus cajan* is a tropical and subtropical legume known as red gram or tur and cultivated for its edible seeds. This crop is damaged by a wide range of insect pests both in the field and storage. Almost all parts of plant are damaged (Upadhyay et al., 1998), and in transitional belt of Karnataka mainly in Dharwad the gall weevil *Alcidodes collaris* (Pascoe) is one of the regular and major pest. This causes 25-30% loss in yield. *Alcidodes collaris* belongs to the subfamily Alcidinae of family Curculionidae and order Coleoptera. Damage is initiated by the female adult by scraping the basal region of seedlings for oviposition. After hatching grubs feed on living tissues inside the stem, and this leads to gall formation at the collar region (Parchabhavi et al., 1972). Due to the gall formation dislodging and drying of seedlings occurs (Rachappa and Lingappa, 2006). In the later stages, adult nibbles the tender shoots which lead to drooping of young tips (Hugar, 2001). Since the pest attacks basal portion of stem and inhabits the soil, its damage goes unnoticed. More importantly, this pest is known to damage early stage of the crop without any visual symptoms which leads to difficulties at farmer's level. There are very few studies on managing the pest, and hence, the present study evaluates management with some insecticides.

MATERIALS AND METHODS

A field experiment was conducted at the Main Agricultural Research Station, Dharwad in pigeonpea in randomized block design with 11 treatments with 3 replications, along with untreated check. Wilt resistant variety TS-3R with the spacing 90x 30 cm was used with plot size of 15 m^2. Sowing was done during 1st week of July, and recommended package of practices were followed except plant protection in the early stage and in the later stage chemical spray was made against *Helicoverpa armigera*. The observations on the total number of plants and the gall formed plants were recorded at 25 and 50 days after emergence, with the plant stand at harvest observed with % reduction in plant stand. Yield was observed and cost economics data were worked out. The data were subjected to statistical analysis to evaluate the statistical significance of treatments.

RESULTS AND DISCUSSION

The results revealed that the least incidence of *A collaris* was observed when seeds were treated with imidacloprid 600FS followed by spray of chlorantraniliprole 18.5SC (T_9) at 20 days after emergence. Maximum reduction in galled plants was also observed with this treatment followed by imidacloprid 600FS seed treatment+ profenophos 50EC (T_3) drenching at 20 days after emergence. Imidacloprid 600FS seed treatment+ chlorpyriphos 20EC drenching was also found effective; and least efficacy was observed with thiamethoxam 35FS seed treatment + fipronil 5SC spray (Table 1). These results corroborate

Table 1. Efficacy of insecticides against *A. collaris* in pigeonpea

S. No.	Treatment	No. of galled plants			% reduction over control	Yield (q/ ha)	Cost of treatment (`/ ha)	Total cost (`/ ha)	Gross return (`/ ha)	Net return (`/ ha)
		25 DAE*	50 DAE*	Mean						
T_1	Imidachloprid 600FS seed treatment 10 ml/ kg of seeds	1.67 (1.46)^{ab}	2.67 (1.77)^{bc}	2.17 (1.63)^{bc}	61.72	18.56 (4.34)^{b}	537.50	37441.50	103936.00	66494.50
T_2	Thiamethoxam 35FS seed treatment 10 ml/ kg of seeds	2.33 (1.68)^{c}	2.67 (1.77)^{bc}	2.50 (1.73)^{c}	55.91	17.96 (4.28)^{b}	81.25	36985.25	100576.00	63590.75
T_3	T_1 + Profenophos 50EC drenching 2 ml/ l at 20 DAE	1.00 (1.22)^{ab}	2.00 (1.56)^{b}	1.50 (1.41)^{b}	73.54	22.24 (4.76)^{ab}	674.30	37578.30	124544.00	86965.70
T_4	T_2 + Profenophos 50EC drenching 2 ml/ l at 20 DAE	1.33 (1.34)^{abc}	3.00 (1.86)^{bc}	2.16 (1.63)^{bc}	61.90	20.53 (4.55)^{abc}	218.05	37122.05	114968.00	77845.50
T_5	T_1 + Fipronil 5SC spray 1 ml/ l at 20 DAE	1.67 (1.46)^{bc}	3.33 (1.94)^{bc}	2.50 (1.73)^{c}	55.91	16.33 (4.08)^{bc}	817.50	37721.50	91448.00	53726.50
T_6	T_2 + Fipronil 5 SC spray 1 ml/ l at 20 DAE	2.00 (1.58)^{c}	3.33 (1.93)^{bc}	2.67 (1.78)^{c}	52.91	15.67 (3.99)^{bc}	361.25	37265.25	87752.00	50486.75
T_7	T1 + Chlorpyriphos 20EC drenching 1.5 ml/ l at 20 DAE	0.67 (1.05)^{a}	2.33 (1.68)^{b}	1.50 (1.41)^{b}	73.54	20.78 (4.59)^{abc}	633.50	37537.50	116368.00	78830.50
T_8	T_2 + Chlorpyriphos 20EC drenching 1.5 ml/ l at 20 DAE	1.33 (1.34)^{abc}	3.33 (1.95)^{bc}	2.33 (1.68)^{bc}	58.91	19.89 (4.49)^{abc}	177.25	37081.25	111384.00	74302.75
T_9	T_1 +Chlorantraniliprole 18.5SC spray 0.2 ml/ llat 20 DAE	0.67 (1.05)^{a}	1.67 (1.46)^{a}	1.17 (1.29)^{a}	79.37	23.38 (4.88)^{a}	1113.50	38017.50	130928.00	92910.50
T_{10}	T_2 + Chlorantraniliprole 18.5SC spray 0.2 ml/lit at 20 DAE	1.67 (1.46)^{bc}	2.33 (1.68)^{b}	2.00 (1.58)^{bc}	64.72	21.02 (4.61)^{abc}	657.25	37561.25	117712.00	80150.75
T_{11}	Untreated check	5.00 (2.34)^{d}	6.33 (2.61)^{d}	5.67 (2.48)^{d}	–	12.89 (3.63)^{c}		36904.00	72184.00	35280.00
	SEm±	0.11	0.14			0.32				
	CD (p=0.05)	0.33	0.40			0.95				
	CV (%)	13.46	12.74			12.77				

*Mean of three replications. Other expenditure = Rs. 36,904; Cost of pigeonpea (Rs./q) = 5600; Cost of chemicals**:** Imidacloprid 600FS = Rs. 430/100 ml; Thiamethaxam 35FS = Rs. 650/ l; Profenophos 50EC = Rs. 600/ l; Chloropyriphos 20EC = Rs. 100/ 250 ml; Fipronil 5SC = Rs. 175/ 100 ml; Chlorantraniliprole 18.5SC = Rs. 180/ 10 ml; Values in parentheses square root transformation; DAE- Days after emergence

with those of Hugar (2001) and Shivaji and Basavana Goud (2003) that chlorpyriphos drenching was superior. As regards yield, pigeon pea seeds treated with imidacloprid 600FS followed by chlorantraniliprole 18.5SC (T_9) spray gave maximum yield of 23.38 q/ha, with highest net return. Imidacloprid 600FS seed treatment + drenching with profenophos 50EC and imidacloprid seed treatment 600FS + drenching with chlorpyriphos 20EC were the next best, while thiamethoxam 35 FS (seed treatment) + fipronil 5SC spray gave the least yield of 15.67 q/ ha (Table 1). Hugar (2001) also reported that drenching with chlorpyriphos gave maximum yield and incremental benefit cost ratio. Shivaji and Basavana Goud (2003) obtained maximum yield with chlorpyriphos drenching. Thus, imidacloprid 600FS seed treatment followed by chlorantraniliprole 18.5SC spray can be recommended for managing gall weevil incidence.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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OCCURRENCE OF THE LITTLE KNOWN DUBIOUS FIVE-RING *YPTHIMA PARASAKRA* ELIOT IN GARHWAL HIMALAYA

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ABSTRACT

A survey carried out from Sankri village to Har-ki-Doon valley in Uttarkashi district, Uttarakhand revealed the occurrence of dubious five ring *Ypthima parasakra* Eliot (1987). The identity of the species was confirmed on examination of its male genitalia and its comparison with its congener, the Himalayan five-ring *Y. sakra* Moore (1958). This is the first site specific record of this little-known species from the Garhwal region of Uttarakhand, Western Himalaya, which is otherwise known from Central and Eastern Himalaya, in India. A brief account on the habitat of the species is given.

Key words: Satyrinae, *Ypthima*, male genitalia, uncus, valva, blue pine, temperate forest, Uttarkashi, Western Himalaya

Ypthima parasakra Eliot (1987) is known to occur as three races from Central to Eastern Himalaya in Nepal, Bhutan, India, adjoining parts of South Tibet and Yunnan in China. The nominate one *Y. p. parasakra* Eliot was described from Central Nepal (type locality Larjung). This subspecies has since then been extensively reported from Mustang, Manang and Lantang districts of Nepal (2155-2677 masl- Eliot, 1987; Smith, 1989; Smith,2006); Bhutan (Eliot, 1987; Gasse, 2013), Sikkim (D'Abrera,1985) and Arunachal Pradesh (Anon.,2021). The second one *Y.p.menpae* Huang was described from Hanmi in South Tibet, China (Huang, 1999), while the third one *Y. p. mabiloa* Huang was also documented from type locality Xiongdang, China (Huang and Wu, 2003). And, *Y. parasakra* remained to be reported from the Western Himalaya (Kehimkar, 2016; Singh and Sondhi, 2016; Sondhi and Kunte, 2018). A male specimen of *Ypthima parasakra parasakra,* was collected on 02.viii.2019 along the Supin river catchment close to Osla in Uttarkashi district, Uttarakhand (31.11453°N; 78.33553°E; 2543 masl) (Fig. 1). The vegetation comprised mostly blue pine *Pinus wallichian*a; deodar *Cedrus deodara*; kharsu oak *Quercus semecarpifolia*; and *Rubus ellipticus, Berberis aristata,* etc. The specimen is kept in Arun P Singh Collection, Forest Research Institute, Dehradun. The identity of the species was confirmed by genitalia dissection. On comparison with its congener Himalayan five-ring *Y. sakra* Moore (1958) from Garhwal (Rose and Sharma,1999). It was observed that both the species were quite similar except that in *Y. parasakra* the 'uncus' was longer and thinner (Eliot, 1987). The 'valva' in *Y. parasakra* is axe-like at the distal end and hooked at the apex, while in *Y. sakra* 'valva' is nearly rounded and blunt at apex (Figs. 2, 3). This record from Western Himalaya is significant as it lies 580 km from Mustang,



a) Ventral view b) Dorsal view

Fig. 1. Male, *Ypthima parasakra*



Fig. 2. Male genitalia of *Y. parasakra*
(UN-Uncus; TEG- Tegumen; AED-Aedeagus; VLV- Valva; SA-Saccus; VIN-Vinculum)

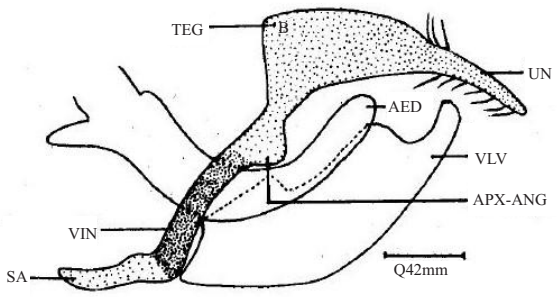


Fig. 3. Male genitalia of *Y. sakra* Moore
(W. Himalaya, Rose and Sharma, 1999)
(UN-Uncus; TEG- Tegumen; AED-Aedeagus; VLV- Valva; SA-Saccus; VIN-Vinculum)

Nepal, the known western limit in the distribution range of this species in the Himalaya.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

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CHIRONOMIDS DIVERSITY OF MANIPUR

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ABSTRACT

Chironomids are Diptera and these are good bioindicators of aquatic habitats, and serve as food of aquatic as well as the lower terrestrial animals. The diversity of these were assessed from the Dhanamanjuri University Campus, which revealed three genera and one unidentified genus viz., *Kiefferulus calligaster*, *Chironomus plumosus*, *Chironomus circumdatus* and one morphotype closely resembling Yama (Yama type). The chromosome compliments of these were observed to be with 2n=8 (*K. calligaster*- AB, CD, EF, G; *C. plumosus*- AB, CD, EF, G; *C. circumdatus*- BF, CD, AE, G and Yama type- AE, BF, CD, G). The differences were in the translocation both in NOR and chromosomes. *Kiefferulus calligaster*, *C. plumosus*, Yama type and *C. circumdatus* were observed coexisting in cemented drainage, with Yama type being predominant in the pond; the latter was mainly collected from more contaminated ditches with domestic waste. The polytene chromosomes were species-specific and could be utilized to identify the species in the larval forms.

Key words: Manipur, Chironomidae, diversity, *Kiefferulus calligaster*, *Chironomus plumosus*, *Chironomus circumdatus*, polytene chromosomes, karyotypes, habitats, ponds, sewage drains

Manipur, the easternmost part of India is a small hilly state, is part of Indo-Burma hotspots of the world (Myers et al., 2000). Chironomids are the most widely distributed and one of the most abundant insects in freshwater in their larval and pupal stage. The chironomids are decomposers and play an important role in the environment. Chironomids can develop in polluted waters such as waste stabilization ponds where they become a dominant macroinvertebrate (Broza et al., 2000) so they are regarded as bioindicators. Chironomids are used in the fields of genetics, cytogenetics, and as food for the fish and amphibian (Sharifian Fard et al., 2014). These also serve as bioindicators of the depredating habitats or pollution (Lucian et al., 2008). Some reports indicate that these are scavengers of toxic substances so not advisable for human consumption (Sharifian Fard et al., 2014). Cytologically the chironomids are arranged descending as AB (1st), CD (2nd), EF (3rd) and G (4th) and specific NOR bearing chromosome is the general rule. From India, 313 chironomid species in 59 genera under 4 subfamilies had been described (Chaudhuri et al., 2001), and only six species are known from the eastern Himalaya of India, and there are no reports from Manipur. This study is an attempt to identify the chironomids and explore their polytene chromosomes and species-specific polymorphism.

MATREIAL AND METHODS

The larvae along with water were collected from two spots located in the front drainage of the Zoology department- spot 1 (E 240 82'08.77", N 930 94'11.87"), spot 2 (E 240 82'07.89", N 930 93'94.16.59") and one spot 3 from the pond (E 240 82'18.20", N 930 93'94.19.89") on the 16th June 2021. Three scoops of the mud and larvae were separately collected. The drain was somewhat clean with the leafy bottom. The larvae were fixed in the 1:3 glacial acidic acid and methanol by volume for 24 hr and transferred to 70% ethanol, while for fresh preparation 70% ethanol was omitted. The material used for polytene studies, head, and tail of the same were made as temporary mount on the same slide. The head and tail features were used for larval identification. The polytene chromosome preparations were done according to Porter and Martin (1977) with slight modifications. The salivary glands were dissected in fixative and stained with 4% acetocarmine solution for 3 min and dipped in fixative for 40 sec and one drop of lactic acid for 1 min or after dissolving the salivary cells. It was observed under coverslip in 10x objective of a compound microscope and photographs were taken using 100x objective with MI 9 prime mobile camera from the eyepiece. The identification of the larvae was done according to Martin (2013, 2016, 2019).

RESULTS AND DISCUSSION

The data given in Fig. 1, reveal that *Kiefferulus* was dominant in the drainages while Yama type was predominant in the pond. These were found attached to leaves, while *Chironomus plumosus* and *C. circumdatus* were attached to stones or on cemented walls of drainage in their igloo-type mud. Larvae of the Yama type were freely swimming or feeding on the bottom of pond and drainage. Larva of *K. calligaster* (Kieffer) showed a light red small (8-10mm) plumosus-type with ventral and lateral tubules. Mentum with fourth laterals was not at the level to fifth laterals (type I), sixth laterals vertical; c2 teeth of the central tooth not well separated, ventromental plate separated medially by at least the width of the median teeth of the mentum, pectin epipharyngis with 15 teeth, premandible is with inner tooth about twice the width of the outer, posterior pair of ventral tubules longer than anterior pair with length and coiled. Lateral tubules well developed (Fig. 2 A, E). *K. calligaster* is common in the Indian subcontinent. The larvae of *C. plumosus* were characterized with well-developed mentum and anal tubules (Fig. 2 B, F). Larvae of *C. circumdatus* were medium plumosus type and 12.25 mm long from 10 IV instar larvae. Mentum with fourth laterals reduced to about the level of fifth laterals (type II), sixth laterals pointed slightly outwards; c2 teeth of the central tooth well separated, ventromental plate separated medially by at least the width of the median teeth of the mentum, pectin epipharyngis with exactly 15 teeth, premandible is with inner tooth about twice the width of the outer, posterior pair of ventral tubules longer than anterior pair with length of 1.95 mm of the anterior while posterior is 3.0 mm and coiled. Lateral tubules well developed (Fig. 2 C, G). A small (7-10 mm) Yama-type with no ventral or lateral tubules, anal tubules in a 'star'-arrangement, posterior prolegs about 4x long than width at base was also observed. Gula and frontoclypeus slightly dark to dark, sometimes with slight darkening outside the frontoclypeus. This may be a difference between the two localities. Clypeal aperture wider at mid-point with a curved ventral border, about 2.5x longer than width at widest point (Fig. 2 D, H). Notable for an unusual larval type (yama-type). This might belong to a new subgenus (http://www.chironomidae.net/Martin/SEAChironfile/SEADSC1.htm).

The diploid number of *K. caligaster* was 8 (2n=8) with three long chromosomes and very short chromosomes. The second chromosome had nucleolus at the one-third of the chromosome. The chromosomes were thick and could be used to test in the laboratory. *K. calligaster* belongs to the cytocomplex "thummi" with chromosome arm combinations: AB CD EF G. (Fig. 2 I); *C. plumosus* had (2n=8) consists of three long chromosomes (chromosomes AB and CD- metacentric, chromosome EF – submetacentric) and one short (chromosome G – acrocentric) (Fig. 1, A-D). It has three Balbiani rings (BRs): one in chromosome arm B and two in chromosome G. The nucleolar organizer (NOR) is localized also in chromosome G; *C. plumosus* belongs to the cytocomplex 'thummi' with chromosome arm combinations: AB CD EF G. The centromere regions of the chromosomes are expressed by thin dark bands in the studied populations (Fig. 2 J). There were four prominent well stained polytene chromosomes in *Chironomus circumdatus*. The polytene chromosomes are typical pseudothummi -cytocomplex combination of BF, CD, AE, and G. The first (B), second (C) and shortest (G) chromosomes were having nucleoli (n). The shortest chromosome is having two prominent Balbiani rings (br) (Fig. 2 K) (courtesy Jon Martin).

Chironomus circumdatus was described by Kieffer (1916) from Formosa (Taiwan); other works include those of Chaudhuri et al. (1992), Kumar and Gupta (1990; cytological aspects), Tripathi et al. (2004). Kumar and Gupta (1990) reported altogether seven inversions comprising six paracentric and one pericentric. The present study reveals that *C. circumdatus* is the common *Chironomus* sp. in Manipur inhabiting fairly uncontaminated aquatic medium. On the basis of homozygous reciprocal translocations species of the genus may have the chromosome arm combination: AB, CD, EF, G or AE, BF, CD, G. Species having the same chromosome arm combination may be united into complexes (Keyl, 1962). So, the karyotype based on polytene chromosomes plays an integrating role in the systematics of the Chironomidae. In these complexes species are differentiated on the basis of homozygous inversions (Keyl 1962). Four relatively long thin polytene chromosomes were observed apparently with the pseudothummi-cytocomplex combinationm AE, BF, CD, G. Arm G with a terminal nucleolus and a BR about

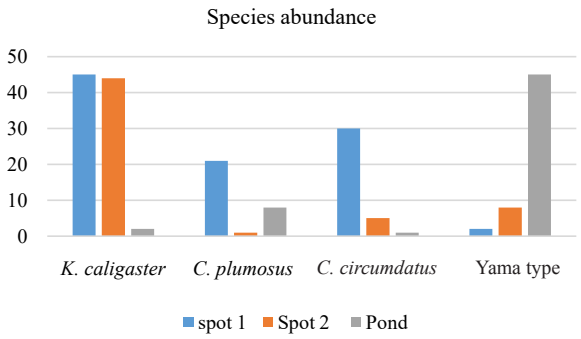


Fig. 1. Comparative species diversity

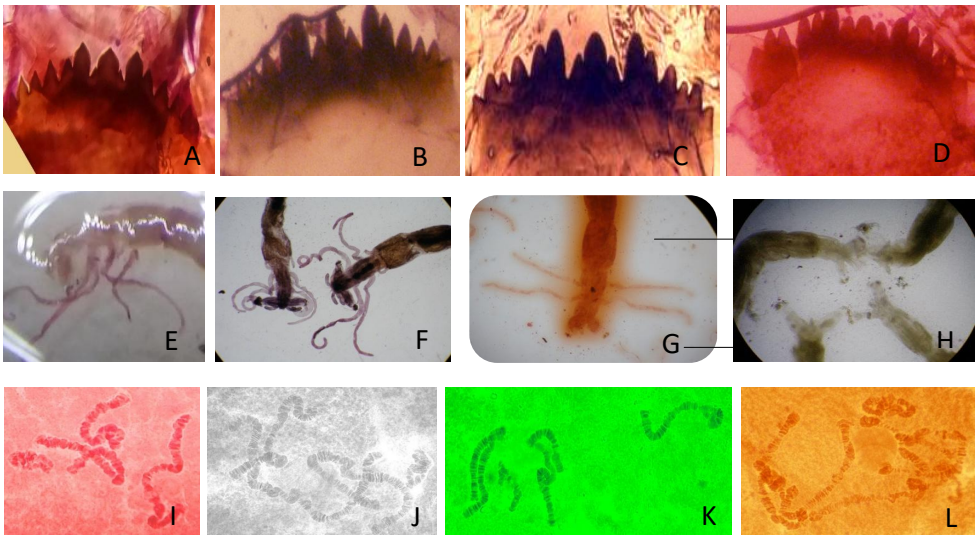


Fig. 2. Mentum, anal tubules and salivary gland polytene chromosomes in larval form of *K. caligaster* (A, E, I), *C. plumosu*s (B, F, J), *C. circumdatus* (C, G, K) and Yama type (D, H, L) respectively. Bar represents 0.5 mm.

a third from the other end (Fig. 2 L). Sublette and Martin (1980) described a new genus Yama, closely related to *Chironomus* with special features of relatively long, rather tanypodine-like posterior prolegs along with a star arrangement of anal tubules (Martin and Chingangbam, 2016). Since then this type is placed as Yama type.

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EVALUATION OF BIOINTENSIVE IPM MODULES AGAINST PESTS OF RABI SORGHUM

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ABSTRACT

Some biointensive IPM modules against sorghum pests explored in the present study revealed that seed treatment with imidacloprid 48%FS @ 12 ml/ kg- cypermethrin @ 2 ml/ l spray at 45 days after emergence (DAE)- treatment T5) and soil application of carbofuran 3G (25 kg/ ha) -seed treatment with chlorpyriphos 20EC @ 5 ml in 20 ml of water- spray of cypermethrin @ 2.0 ml/ l at 25DAE (treatment T5- Recommended package of practices of UAS, Dharwad) were significant and on par against shoot fly *Atherigona soccata* (Rondani) (13.70 and 14.59% deadhearts), aphid (*Rhopalosiphum maidis* (Fitch) (20.94 and 17.36 aphids/ 5cm^2 leaf) and shoot bugs (*Peregrinus maidis* Ashmead) (9.77 and 10.62 shoot bugs/ plant). In case of stem borer *Chilo partellus* (Swinhoe) incidence was less with treatment T6: soil application of carbofuran 3G (25 kg/ ha)- seed treatment with chlorpyriphos 20EC @ 5 ml in 20 ml of water spray of cypermethrin @ 2.0 ml/ l at 25 DAE (3.43% deadhearts) followed by T1 (seed treatment with imidacloprid 48%FS @ 12 ml/ kg + intercropping with chickpea: sorghum (4:2)- NSKE (5%) spray at 45 DAE). The yields were superior with T5 and T6 (2200 and 2317 kg/ ha, respectively).

Key words: Sorghum, azospirillum, *Trichoderma*, vermicompost, intercropping, *Lecanicillium lecanii*, *Atherigona soccata*, *Rhopalosiphum maidis*, *Peregrinus maidis*, seed treatment, cypermethrin, imidacloprid, yield

Sorghum in India is an important cereal crop cultivated in warmer climates, with most of its varieties drought and heat-tolerant (Satyagopal et al., 2014). But, it is infested with about 150 insect pests (Guo et al., 2011), and of these about 10 to 12 cause 12 to 83% avoidable damage (Premkishore, 1987). Among these pests, shoot fly *Atherigona soccata* (Rondani) causes yield losses up to 90% (Jotwani and Srivastava, 1970). Rensburg and Hamberg (1976) highlighted severity of aphid damage causing 77% reduction in grain yield' and shoot bug in India causes 41% yield loss (Hosmani and Chittapur, 1997). The term IPM earlier used as 'integrated control' by Bartlett (Bartlett, 1956) and was further elaborated by Stern and co-workers (Stern et al., 1959). Several crop management practices were out but among them IPM provides ecofriendly management with beneficial yield to farmers. This study focuses on few biointensive IPM against sorghum pests.

MATERIALS AND METHODS

The field experiment was laid out during rabi 2016-17 and 2017-18 at the Regional Agricultural Research Station (RARS), Vijayapur, Karnataka, India. Sowing was done at second fortnight of September following package of practice recommended except plant protection. The experiment was laid out in randomized block design with seven treatments replicated thrice (for details see Table 1). The incidence of *A. soccata*, aphid *Rhopalosiphum maidis* (Fitch), shoot bug *Peregrinus maidis* Ashmead and stem borer *Chilo partellus* (Swinhoe) were evaluated following standard procedure (Sharma et al., 2008). The deadhearts due to *A. soccata* was recorded at 28 days after emergence (DAE) by counting the total number of plants and plants with deadheart symptoms; while that of *C. partellus* was recorded at 45 DAE. The incidence of *R. maidis* was observed as number of aphids/ sq.cm leaf area on five randomly selected plants (3 leaves/ plant i.e. top, middle, bottom leaves), and rated in 0-9 scale; while *P. maidis* was observed from five randomly selected plants and rated using 0-9 scale. The data were subjected to ANOVA and the treatment means were compared by DMRT (Gomez and Gomez, 1976).

RESULTS AND DISCUSSION

The results revealed the IPM module T_6 (soil application of carbofuran 3G 25 kg/ ha-seed treatment with chlorpyriphos 20EC @ 5 ml in 20 ml of water-spray of cypermethrin @2.0 ml/ l at 25 days after emergence (DAE) (RPP of UAS, Dharwad) gave best results against *A. soccata* (14.59% deadhearts), *C. partellus* (3.43% deadhearts), *R. maidis* (17.36/ 5 cm^2 leaf) and *P. maidis* (10.62/ plant). This treatment was on par with the module T_5 (seed treatment with

Table 1. Efficacy of BIPM modules in rabi sorghum (2016-17, 2017-18, pooled data)

Treatment No.	Treatments	*A. soccata* deadheart (%)	*C. partellus* deadheart (%)	*R. maidis*/ 5 cm^2 /leaf	*P. maidis*/ plant	Crop yield (kg/ ha)
T_1	Seed treatment with imidacloprid 48% FS @ 12ml/kg + intercropping with chickpea: sorghum (4:2)- NSKE (5%) spray at 45DAE	15.07 (3.94)	3.62 (2.03)	35.89 (6.02)	12.80 (3.64)	2171
T_2	Seed treatment with biofertlizers (*Trichoderma*+ PSB + Azospirillum) + 50% neem cake (1q/ha) +50% + vermicompost (4q/ha) at the time of sowing- NSKE (5%) spray at 45DAE	19.66 (4.47)	6.00 (2.55)	27.54 (5.29)	14.53 (3.87)	2095
T_3	Seed treatment with biofertlizers (*Trichoderma*+ PSB + Azospirillum) + Btk @ 2g/lt at 25 DAE -Spray of *Lecanicillium lecani* @ 2ml/l at 45DAE	19.30 (4.45)	4.31 (2.19)	30.94 (5.61)	16.95 (4.17)	2213
T_4	Seed treatment with imidacloprid 48% FS @ 12ml/kg + eco feast crop like one row of cowpea around sorghum - NSKE (5%) spray at 45DAE	15.58 (4.00)	4.19 (2.14)	29.81 (5.50)	12.84 (3.65)	2175
T_5	Seed treatment with imidacloprid 48% FS @ 12ml/kg- cypermethrin @ 2ml/l spray at 45DAE	13.70 (3.77)	4.34 (2.19)	20.94 (4.63)	9.77 (3.20)	2200
T_6	Soil application of carbofuran 3G (25kg/ha) -seed treatment with chlorpyriphos 20EC @ 5ml in 20ml of water- spray of cypermethrin @ 2.0ml/l at 25DAE (RPP of UAS, Dharwad)	14.59 (3.88)	3.43 (1.98)	17.36 (4.22)	10.62 (3.32)	2317
T_7	Farmer practice (Untreated)	23.19 (4.86)	6.68 (2.67)	52.58 (7.32)	21.54 (4.69)	1860
	CD (p=0.05)	0.71	0.41	1.09	0.76	400.0
	S Em ±	0.24	0.13	0.36	0.25	132.0
	CV (%)	10.02	10.39	11.31	11.45	10.58

imidacloprid 48%FS @ 12 ml/ kg- cypermethrin @ 2ml/ l spray at 45DAE) except for *C. partellus*, which was found to be less (3.62% dead hearts) with treatment T_1 (seed treatment with imidacloprid 48%FS @ 12ml/kg + intercropping with chickpea: sorghum (4:2)- NSKE (5%) spray at 45DAE) (Table 1). These results corroborate with those of Balikai (1998; 1999) and Balikai and Lingappa (2003) who observed that seed treatment with 10 g imidacloprid 70 WS/ 100 g seed was the most effective against *A. soccata* and aphids. Karabhantanal et al. (2018) also observed that the module with imidacloprid 70 WS @ 3 g/ kg seed significantly reduced *A. soccata,* aphids (13.85/sq. cm) and shoot bugs. The yields obtained was maximum with T_6: soil application of carbofuran 3G (25 kg/ ha) -seed treatment with chlorpyriphos 20EC @ 5 ml in 20 ml of water- spray of cypermethrin @ 2.0 ml/ l at 25DAE (RPP of UAS, Dharwad) (2317 kg/ ha). These results are similar to Karabhantanal et al. (2018) and Balikai (1998, 1999) with seeds treated with imidacloprid 70 WS @ 3 g/ kg seed at 45 DAE. Thus, maximum yield can be obtained with soil application of carbofuran 3G (25 kg/ ha) -seed treatment with chlorpyriphos 20EC @ 5 ml in 20 ml of water- spray of cypermethrin @ 2.0ml/l at 25DAE (RPP of UAS, Dharwad).

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AUTHOR CONTRIBUTION STATEMENT

Karabhantanal designed and conducted experiments. Dharavath analyzed data and wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

Authors clearly state that there does not exist any conflict of interest.

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FEEDING BEHAVIOUR OF *COCCINELLA SEPTEMPUNCTATA* AND *COCCINELLA TRANSVERSALIS*

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ABSTRACT

The mass production of coccinellids at the Biocontrol Laboratory of Chandra Shekhar Azad University of Agriculture and Technology, Kanpur included laboratory studies on the predatory behaviour of *Coccinella septempunctata* L. and *C. transversalis* (F.) (Coleoptera: Coccinellidae). The study was done with the natural food, *Lipaphis erysimi* and *Brevicoryne brassicae* (Hemiptera: Aphididae). The predatory efficiency of the larvae and adults was determined by quantitative method of aphids consumed. The results revealed that the feeding rate increased gradually from the first to fourth larval instar, irrespective of the aphid host, with the first instars being less voracious than later ones.

Key words: *Coccinella septempunctata*, *C. transversalis*, *Lipaphis erysimi*, *Brevicoryne brassicae*, life stages, feeding behaviour, predatory efficiency, larval instars, adults

Insect pests have always been a threat to agricultural productivity. Various chemicals (pesticides) are applied against insect pests to counter this (Pearson, 2004). The disadvantages of pesticides call for a safe and cheap methods of IPM which ensures environmental safety (Solangi, 2004). Ladybird beetles (Coccinellidae: Coleoptera) are important predators in natural and agricultural habitats and prey upon many economically important pests, including aphids, mealy bugs, scale insects, thrips, leafhoppers, mites, and other soft bodied insects (Dixon 2000; Khan et al. 2009, Takizawa et al*.,* 2000; Devi et al*.,* 2002). These are predators in both adult and larval phases (Gilkeson 2001; Oliveira et al., 2004; Cotrell 2005), and these feed on aphids or coccids, with a few feeding on both types of prey (Weber and Lundgren 2009). Such biological control performed by naturally occurring predators and parasitoids can have considerable impacts on aphids (Smith and Chaney, 2007; Bowie et al. 1999). Targeted release of such biocontrol agents against aphids in vegetable crops is widely used in greenhouse vegetable production (Rabasse and van Steenis, 1999; Zehnder et al*.*, 2007). This study explores the predatory feeding behaviour of two such coccinellids.

MATERIALS AND METHODS

Adults of *C. septumpunctata* and *C. transversalis* were collected from different crops and brought to the laboratory. Pairs of beetles were placed in duly covered rearing jars with sufficient number of aphids as food and substrate for egg laying. To rear the predator coccinellid, aphid colonies were maintained in cages in the laboratory on fresh leaves of mustard and cabbage. Mass cultures were initiated by collecting newly emerged adults and maintained in plastic jars (30x 15 cm), with an abundant supply of prey from the aphid colonies until oviposition. The rearing jars were provided with wrinkled paper to act as oviposition sites. The eggs laid were collected every 24 hr, transferred to petridishes, and allowed to hatch. The cultures were maintained at 25± 2°C and 65 ± 5% RH, from which emerged larvae were taken and fed with aphids, the emerginf adults from this stock were sexed, and the eggs were incubated. The 1st instar grubs were used for the studies. Newly emerged larvae were fed separately with 3rd or 4th instar nymphs of the aphids *Lipaphis erysimi, Brevicoryne brassicae*, which were kept fed on leaves of the respective host plants in petridishes. Each treatment was replicated 10 times with at least five individuals/ replication.

RESULTS AND DISCUSSION

Laboratory studies on the predatory behaviour of *C. septumpunctata* and *C. transversalis* on *L. erysimi* and *B. brassicae* revealed that the 4th instar larva consumed significantly more aphids. The behaviour estimated by the quantitative method of aphids consumed by each larval instars and adults revealed that the feeing rate increased gradually from the first to fourth instar, irrespective of the aphid host; the predation rate showed

Table 1. Feeding behaviour of coccinellid on aphids under laboratory conditions

S.No.	Insect stages (Predator)	*Coccinella septumpunctata*		*Coccinella transversalis*	
		Consumed *L. erysimi* (No.)	Consumed *B. brassicae* (No.)	Consumed *L. erysimi* (No.)	Consumed *B. brassicae* (No.)
1.	1st instar grub	16.33 (4.102)*	14.00 (3.807)	14.33 (3.850)	17.00 (4.183)
2.	2nd instar grub	23.33 (4.881)	17.66 (4.261)	21.0 (4.636)	19.00 (4.415)
3.	3rd instar grub	30.66 (5.580)	23.66 (4.915)	24.33 (4.982)	22.67 (4.813)
4.	4th instar grub	36.33 (6.068)	31.33 (5.641)	30.0 (5.522)	30.66 (5.582)
5.	Adult	40.33 (6.389)	36.66 (6.095)	34.33 (5.901)	32.00 (5.700)
	Total consumed/ one coccinellid	146.98 (12.144)	123.31 (11.126)	123.99 (11.157)	121.33 (121.33)
	CD (p=0.05)	0.139	0.293	0.210	0.257
	SE (m)	0.042	0.088	0.063	0.077

*Figures in parentheses square root transformed $\sqrt{X + 0.5}$

that *C. septumpunctata* consumed 146.98 aphids of *L. erysimi* and 123.31 aphids of *B. brassicae* (Table 1). The larvae and adult of *C. transversalis* consumed 123.99 of *L. erysimi* and 121.33 of *B. brassicae*. The aphids consumed/ day by the first instar larvae was less than the later ones, and the first and second instars preferred young nymphs compared to older nymphs and adults. Preference for young nymphs may be due to their smaller size and lower numbers of encounters (Dixon and Russel, 1972). The fourth instar larvae of *C. transversalis* consumed more aphids compared to adult female (65.67 and 62.17, respectively). Pandey and Khan (2002) observed that the fourth instar larvae of C. *septempunctata* consumed more aphids (*Myzus persicae*) than did the adults. Less consumption of *Aphis craccivora* was observed followed by *B. brassicae*; these findings of Jonathan (2005) and Maharajan (2018) support the present results. Wagle et al. (2006) observed that *C. transversalis* devoured 38.8 aphids of *B. brassicae/* day. Veeravel and Baskaran (1996) and Malik et al. (1998) showed that an adult of *C. transversalis* consumed 67.66 aphids/ day. The feeding potential of *C. septempunctata* and *Menochilus sexmaculatus* (F.) was studied on *L. erysimi* (Shenmar and Brar, 1995).

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AUTHOR CONTRIBUTION STATEMENT

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FIELD EVALUATION OF RED PALM WEEVIL *RHYNCHOPHORUS FERRUGINEUS* OLIVIER PHEROMONE LURES

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ABSTRACT

The red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) is a key pest of coconut. The efficiency of pheromone lures sourced from three countries along with natural (sugarcane) and synthetic (ethyl acetate) co-attractants, was tested in the infested coconut plantation. The results revealed that synergists and co-attractants are essential to sustain trapping efficiency. The best weevil captures were obtained when the pheromone trap had both the natural food bait (sugarcane) and the synthetic co-attractant (ethyl acetate). Pheromone traps with only ethyl acetate as a co-attractant led to lower captures as compared to pheromone traps with only the food bait (sugarcane). In general, the captures were female dominant with an overall male: female ratio of 1:1.35.

Key words: *Rhynchophorus ferrugineus*, ferrugineol, co-attractants, pheromone traps, coconut palm, sugarcane, ethyl acetate, synergist, trap captures, male female ratio

Coconut palm (*Cocos nucifera* L.) occupies a dominant role among the cultivated palm species in India as it provides livelihood securities >10 million people in India. The crop is cultivated in an area of 2.153 million ha with a total production of 21308 million nuts (CDB, 2020). The red palm weevil (RPW) *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) also known as the Asian palm weevil is a key pest of palms (Arecaceae) in diverse agroecosystems. Globally RPW is reported from 50 countries infecting 40 palm species (Faleiro et al., 2020). Juvenile palms (5-15 years old) are more vulnerable to infestation (Chandrika Mohan et al., 2020). Being an internal tissue feeder, the symptoms are seen at advanced stage of infestation, and hence, early detection/ monitoring of pest is important in IPM approach. Semiochemical mediated control of *R. ferrugineus* is currently confined to the use of food baited pheromone traps (Soroker et al., 2015; Oehchlager, 2016). A good lure would capture more weevils and retain trapping efficiency for long (Faleiro et al., 1999). Fermenting dates, sugarcane, molasses, fresh coconut petiole bits are some of the commonly used food baits in RPW pheromone traps (Faleiro et al., 2020). Ethyl acetate (EA) when incorporated in pheromone traps enhances weevil captures (Oehchlager, 2016). Al-Saoud (2013) reported that black colour pheromone traps along with EA would significantly enhance the efficiency of the mass-trapping programme. In the present study, three RPW pheromone lures from USA, UK and Costa Rica were evaluated for attracting the *R. ferrugineus* along with natural (sugarcane bits) and synthetic (EA) co-attractants.

MATERIALS AND METHODS

The experiment was conducted in *R. ferrugineus* infested coconut plantation at the farm of Don Bosco College of Agriculture, Sulcorna, Quepem Goa, India (15.1060^0 N, 74.1486^0 E) from March 2 to 4 May, 2021. Pheromone traps were fabricated as described in Faleiro (2006) using a 5 l size polypropylene bucket with four uniform rectangular windows (10x 2 cm) cut equidistantly below the bucket rim. The bucket was covered with a lid which was secured to the bucket with wire. The pheromone lure and ethyl acetate dispensers were hung to the lid with a piece of wire so as to firmly remain inside the bucket. The outer surface of the bucket was covered with nylon mesh to facilitate weevils to climb and enter the trap. Buckets were painted black, as black coloured *R. ferrugineus* traps are reported to record significantly higher weevil captures (Al-Saoud, 2013). Main treatment (factor A) comprised of *R. ferrugineus* pheromone (ferrugineol) lures from three countries viz., IT189™ by ISCA Technologies, USA, non-commercial RPW lure by IPS, UK and Ferrolure+™ by Chemtica International, Costa Rica. All three lures are ferrugineol based formulations. While the Chemtica lure is a liquid dispensed through a plastic sachet, the other two lures by ISCA and IPS are wax based capsules.

The subtreatment (factor B) comprised of three treatments including natural (sugarcane bits), synthetic (ethyl acetate) kairomone producing synergist and Combination of both. All treatments contained 1 l water in the bucket trap. The food bait was mixed in the water. One gram carbofuran 3G was added in the water to kill captured weevils and prevent escapes. Where ever applicable, 100 g of sugarcane bits and 10ml ethyl acetate (Ethyl acetate 99.5%, LOBA CHEMIE PVT.LTD.) was incorporated to the trap. While the sugarcane bits were mixed with the water in the trap, 10 ml EA was dispensed in BOROSILR glass vials with a 1mm perforation in the lid of the vial to maintain a release of about 1ml EA/day. Treatment combinations involving *R. ferrugineus* pheromone lures and kairomone producing synergists are as follows: 1. A1B1: ISCA lure (IT189 ™)+ Food bait, 2.A1B2: ISCA lure (IT189 ™)+ Ethyl acetate, 3. A1B3: ISCA lure (IT189 ™) + Ethyl acetate +Food bait, 4. A2B1: IPS lure+ Food bait, 5. A2B2: IPS lure+ Ethyl acetate, 6. A2B3: IPS lure + Ethyl acetate + Food bait, 7. A3B1: Chemtica International lure (Ferrolure+™)+ Food bait, 8. A3B2: Chemtica International lure (Ferrolure+ ™)+ Ethyl acetate and 9. A3B3: Chemtica International lure (Ferrolure+ ™)+ Ethyl acetate+ Food bait.

Treatment traps were set on the ground under the shade of palm canopy about 20m apart in each block. The trial was replicated thrice. R. ferrugineus population is known to be aggregated in nature (Faleiro et al., 2002) which could lead to certain spots in the field capturing more weevils. In order to neutralize treatment bias if any due to spot effect, treatments were allowed to remain at a given spot in the field for 7 days and moved sequentially from one spot to another every week, ensuring that each treatment was placed at every spot in the field for 7 days. Treatment traps were serviced once a week when the food bait, water and insecticide in the bucket traps was renewed and also the EA in the glass vials was replenished. During trap servicing observations on weevil captures gender wise was recorded. The field trial was conducted as a two-factor factorial experiment. The nine treatment combinations in the trial were replicated thrice as indicated above. Treatments within each block were set 20m apart, while a distance of 100m was maintained between replications. A weekly record of the number of weevils trapped in the pheromone traps was maintained gender wise for the study period (2 March- 4 May, 2021). Data on weevil captures recorded every week was compiled at the end of the experiment and subjected to statistical analysis (ANOVA) using the Web Agri Stat Package (WASP1), available at https://ccari.res.in/wasp/index.php

RESULT AND DISCUSSIONS

Results presented in Table 1 reveal that the three pheromone lures (factor-A) tested were statistically at par with regard to the mean weevils trapped which ranged from 3.15 to 3.96 weevils captured/trap/week. As regards the co-attractants (factor-B) evaluated, the best synergistic effect of 6.88 weevils/trap/week was seen when ethyl acetate and the food bait (sugarcane) was incorporated together in the *R. ferrugineus* pheromone traps and was significantly superior in RPW pheromone traps with the food bait (2.75 weevils/ trap/ week) and also with pheromone traps containing EA without the food bait (1.19 weevils/ trap/ week). Further, Table 1 reveals significant interaction effects between the pheromone lure and the co-attractants. The best weevil captures were recorded when the pheromone trap had both the natural food bait (sugarcane) and the synthetic co-attractant (ethyl acetate). Pheromone traps with only EA as a co-attractant recorded lower captures as compared to pheromone traps with only the food bait. In general, the captures were female dominant with an overall male: female ratio of 1:1.35.

Results presented above are in agreement with previous findings. While *R. ferrugineus* pheromone lures may not significantly differ in their attraction to the pest, co-attractants incorporated in pheromone traps are known to play an important role in synergising the efficiency of the lure and enhancing captures. Hallett et al. (1999) first highlighted the importance of adding a food bait to *R. ferrugineus* pheromone traps. Subsequently several workers emphasized the importance of adding food baits (Faleiro and Chellapan, 1999; Hallett et. al., 1999; Nair et. al., 2000). Dates (khajur) when used in the pheromone traps gave the highest captures, which was at par with sugarcane and significantly superior to coconut petiole (Faleiro and Satarkar, 2005). Ethyl acetate when added to food baited *R. ferrugineus* pheromone traps is known to enhance weevil captures by a factor of 2 to 5 (Vacas et al., 2014; Oehlschlager, 2016; Al-Saroj et al., 2017; Amzah and Baki, 2020) and is in agreement with the findings of this study. This study could potentially lead to the development of dry traps that do not need servicing like the Electrap™ (Al-Saroj et al., 2017). Although dry trapping is advancement in *R. ferrugineus* trapping, the need to periodically visit the trap in the field for recording data on weevil captures is another

Table 1. Mean weevil captures in *R. ferrugineus* pheromone traps baited with natural and synthetic co-attractants

No.	Treatment	Mean weevil Captures (catch/ trap/ week)
Factor-A: Pheromone lures		
1	ISCA lure	3.15
2	IPS lure	3.96
3	Chemtica lure	3.70
CD (p=0.05)		NS
Factor-B: Natural and Synthetic Synergists (Sugarcane/ Ethyl acetate)		
1	Food bait (sugarcane)	2.75
2	Ethyl acetate	1.19
3	Ethyl acetate + food bait	6.88
CD (p=0.05)		0.96
Interaction (Factor AxB): Lure x Bait		
1	ISCAlure + Food bait	2.26 (1:1.22)
2	ISCA lure + Ethyl acetate	1.48 (1:1.35)
3	ISCA lure + Ethyl acetate + Food bait	5.71 (1:1.08)
4	IPS lure + Food bait	3.18 (1:3.10)
5	IPS lure + Ethyl acetate	1.19 (1:2.20)
6	IPS lure + Ethyl acetate + Food bait	7.52 (1:1.14)
7	Chemtica lure + Food bait	2.81 (1:0.94)
8	Chemtica lure + Ethyl acetate	0.89 (1:1.14)
9	Chemtica lure + Ethyl acetate + Food Bait	7.40 (1:1.27)
CD (p=0.05) /sex ratio		1.67 (1:1.35)

NS: Difference between mean values not significant; Figures in parentheses sex ratio (male: female) indicating number of female weevils captured for every male weevil trapped.

bottleneck especially in area-wide operations. In this context Spotta UK, has developed a prototype of a smart *R. ferrugineus* pheromone trap that not only eliminates servicing, but also records and automatically transmits weevil capture data on a 24x 7 basis (https://blog-spotta.co/2021-red-palm-weevils-detection-trials). Table 1 also reveals female dominant captures in the trial, which is in agreement with previous findings and good for *R. ferrugineus* control as female weevils trapped in *R. ferrugineus* traps are known to be young, gravid and fertile (Faleiro et al., 2003). Capture of such female weevils would mitigate buildup of the population. Sugarcane bits when added together with ethyl acetate in pheromone traps significantly enhance weevil captures, and addition of ethyl acetate alone could lead to the development of dry traps that do not need periodic servicing.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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INTEGRATED MANAGEMENT OF RODENT PESTS IN SUGARCANE USING RODENTICIDES AND ANTIFERTILTY AGENT TRIPTOLIDE

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ABSTRACT

Five blocks of farmer's sugarcane fields were selected in district Jalandhar, Punjab during December 2016 to February 2017. These blocks were treated with 2% zinc phosphide (ZnP), 2% ZnP+ 0.005% bromadiolone (Br), 2% ZnP+ 0.25% triptolide (Tr) and 2% ZnP+ 0.005% Br+ 0.25% Tr at cane maturation stage; and 5th block was kept as untreated control. The post census and % re-buildup of rodents after 30 and 45 days of Tr treatment and cut canes (%) were significantly less in rodenticides+ Tr treated blocks (ZnP+ Tr and ZnP+ Br+ Tr) as compared to blocks treated only with rodenticides (ZnP alone and ZnP + Br). However, burrows and yield loss were found to be significantly less with ZnP+ Br+ Tr treatment. Thus, antifertilty agent, triptolide can be used along with rodenticides for better control of rodents in sugarcane.

Key words: Bromadiolone, zinc phosphide, triptolide, rodents, rebuildup, antifertility agent, burrow count, sugarcane, cane maturation, cut canes, damage, yield loss

Sugarcane (*Saccharum officinarum*) is an important cash and industrial crop, and occupied 91000 ha in Punjab during 2019-20 with yield of about 735 q/ ha (Anonymous, 2021). Sugarcane being a long duration crop provides an ideal habitat for a complex of rodents. There are about 103 species and 89 subspecies of rodents reported in India (Pradhan and Talmale, 2011), with serious economic loss (Singla and Babbar, 2010). Rodent damage to sugarcane has always been a matter of economic concern as 19.12% damage is caused in Punjab (Singla and Parshad, 2010). After use of rodenticides and other methods, rodents rapidly rebuild their population by enhancing their reproduction (Shilova and Tchabovsky, 2009), and repeated use of rodenticides, which cause several problems like bait shyness, resistance and other non-target toxicity hazards (Mineau et al., 2004). Fertility control has been identified as a more appropriate and environmentally safe strategy (Zhang et al., 2009). Witmer (2019) reported that it is essential to improve the effectiveness and safety of rodenticides. Triptolide is one of the registered sterilants for rodent management in China (Huang, 2014). IPM strategy through a combination of techniques such as biological control, habitat manipulation, modification of cultural practices, introduction of resistant varieties offers promise (Amir and Raza, 2020). The present study aims at a strategy for IPM of rodents in sugarcane deploying chemical and biological methods using rodenticides in combination with triptolide at cane maturation stage.

MATERIALS AND METHODS

Five blocks (I-V) of farmer's sugarcane fields each further consisting of three replicated fields of 0.40 ha were selected at village Apra (31.0861°N, 75.8781°E), Nangal (31.3832°N, 76.3766°E) and Thalla (31.0513°N, 75.8614°E) of district Jalandhar, Punjab during December, 2016 to February, 2017. The pre and post census bait consumption was recorded from all these keeping 1 kg/ ha of the plain WSO bait (cracked wheat, powdered sugar and groundnut oil in the ratio of 96:2:2) on pieces of paper at 20 bait points/ field arranged in a grid and by collecting and weighing remaining bait on third day to determine the bait consumption (g/ 100g bait). Precensus rodent population was thus assessed, and these fields at cane maturation stage were treated with 2% zinc phosphide (ZnP) followed by 0.005% bromadiolone- Br, and 0.25% triptolide (Tr) by keeping different baits @ 1 kg bait/ ha at 100 bait points arranged in a grid as: Block I: 2 % ZnP only; Block II: 2% ZnP+ 0.005% Br after 15 days; Block III: 2% ZnP + 0.25% triptolide (Tr) after 15 days; Block IV: 2% ZnP+ 0.005% Br after 15 days, and 0.25% Tr bait after another 15 days; and Block V: untreated control. The post- census (I) bait consumption was taken from all the fields after 30 days of treatment with 2%ZnP+ 2% ZnP+ 0.005% Br in all blocks. Similarly, post census II, III and IV bait consumption for second, third and fourth time respectively was taken in all blocks after 15, 30 and 45 days of treatment with Tr. From these pre and first post census bait consumptions,

Table 1. Effect of treatments on bait consumption, control success, rodent burrows, cut canes and yield loss in sugarcane crop (December 2016-February, 2017

Block (n=3 each)	Pre-census	Plain bait consumption (g/ 100g) Post-census				Control success (%)		No. of rodent burrows/ acre	Cut canes (%)	Yield loss (Q/acre)
		I	II	III	IV	wrt same field	wrt control field			
I (ZnP)	78.67± 0.88^{a}	50.67± 0.67^{a}	58.33± 4.05^{a}	75.33± 2.91^{a}	83.33± 1.86^{a}	35.59±	37.15±	12.67±	2.51±	0.20±
			(6.67± 1.86^{a})^{I}	(24.67± 2.91^{a})^{II}	(32.67± 2.18^{a})^{III}	0.56^{a}	2.56^{a}	0.67^{a}	0.36^{a}	0.02^{a}
II (ZnP+Br)	79.00± 2.30^{a}	42.67± 2.18^{a}	50.0± 0.58^{a}	82.00± 2.52^{a}	78.67± 3.33^{a}	45.75±	45.02±	12.33±	2.69±	0.15±
			(6.67± 0.67^{a}) ^{I}	(24.00± 3.61^{a}) ^{II}	(36.00± 3.21^{a})^{III}	4.34^{a}	4.40^{a}	1.33^{a}	0.70^{a}	0.04^{a}
III (ZnP+Tr)	81.67± 0.88^{a}	54.67± 2.03^{a}	61.33± 1.46^{a}	63.67± 6.84^{b}	73.67± 5.84^{b}	33.02±	32.00±	12.00±	1.35±	0.15±
			(5.33± 1.20^{b}) ^{I}	(21.00± 6.43^{a})^{II}	(19.00± 7.55^{b})^{III}	2.82^{a}	2.73^{a}	2.00^{a}	0.50^{b}	0.05^{a}
IV (ZnP+Br+Tr)	83.33± 1.20^{a}	58.00± 2.89^{a}	63.33± 4.05^{a}	65.67± 2.02^{b}	72.33± 1.45^{b}	30.35±	29.42±	6.33±	1.00±	0.10±
			(4.67± 0.88^{a}) ^{I}	(10.00± 2.31^{b})^{II}	(14.33± 3.93^{b})^{III}	3.78^{a}	3.83^{a}	0.33^{b}	0.19^{b}	0.03^{b}
V (Untreated control)	76.00± 0.58^{a}	77.33± 1.45^{b}	83.33 .± 1.86^{b}	85.00± 1.53^{c}	95.33± 1.33^{c}	--	--	38.67±	4.01±	1.50±
			(7.67± 3.53^{a}) ^{I}	(7.67± 2.90^{b})^{II}	(18.00± 0.58^{b})^{III}			1.76^{c}	0.71^{c}	0.07^{c}

Values mean ± SE; Values with superscripts a-c and I-III in a column and a row respectively differ significantly at p≤0.05; Figures in parentheses indicate values for population re-buildup

efficacy of rodenticides was evaluated by determining reduction in rodent activityin all the treated blocks with respect to the same field as well with respect to untreated control fields following Singla and Parshad (2010). The difference in post census II, III and IV bait consumption data taken after 15, 30 and 45 days of Tr treatment gave the % rebuildup, calculated as described by Dhar et al. (2014). Rodent pest live burrow count was determined at preharvest stage on the basis of characteristic burrow entrances (Dubey, 2001; Neelanarayan, 2004). The cut canes (%) and yield loss (q/ ha) depicting preharvest rodent damage were calculated following Singla and Parshad (2010) and Singla and Babbar (2012). The data was interpreted as Mean± SE and subjected to paired Student's t-test and one way ANOVA (p=0.05).

RESULTS AND DISCUSSION

The sugarcane fields in all blocks were surrounded by wheat and potato crops. The precensus bait consumption by rodents in fields of blocks I to V varied non-significantly (Table 1). The post census counts revealed a significantly low bait consumption in blocks I, II, III and IV as compared to untreated control block V, with a non-significant difference among treated blocks; in triptolide treated blocks III and IV was found to be significantly low. This indicated that combination of rodenticides and triptolide was effective. The rodent control success varied non-significantly among different blocks with respect to same (30.35- 45.75) as well as control (29.42- 45.02). The control success with rodenticides i.e. ZnP or ZnP+Br are in accordance with the earlier studies in sugarcane. Singla and Babbar (2012) found that three rodenticide treatments, first in July and second in October–November with two rodenticide baitings i.e. ZnP followed by Br each at 15 days interval and third treatment in December–January with single baiting of 0.005% Br was effective. Singla and Parshad (2010) observed double-baiting with ZnP and Br resulted in significant reduction in rodent activity. The present studies revealed significantly less re-buildup of rodents in block IV (ZnP+ Br+ Tr) and in blocks III (ZnP+ Tr) as well as IV (ZnP+ Br+ Tr) after 30 and 45 days of Tr treatment, respectively. Dhar et al. (2014) also suggested potential of Tr treatment in sugarcane in integration with chemical control. Laboratory studies also revealed the antifertility potential of Tr against both the male and female *Bandicota bengalensis* (Dhar and Singla, 2013 and 2014). The inhibitory action of Tr on testicular germ cells and ovarian follicular cells led to inhibition of reproduction (Deng et al., 2011; Dhar and Singla, 2013 and 2014).

The number of rodent burrows was found to be significantly less in block IV as compared to other treated blocks (block I,II and III); this indicated better control with ZnP+ Br+ Tr. *Bandicota bengalensis* was found to be the predominant rodent species followed by *Mus booduga* and *Millardia meltada* in all the blocks. Sugarcane crops were found to be infested with *B. bengalensis*, *M. booduga*, *M. meltada* and *Golunda ellioti* with *B. bengalensis* being the most prevalent (Singla and Parshad, 2010; Singla and Babbar, 2012). Qamar et al. (2019) reported that *B. bengalensis* eat the stem of sugarcane especially from internodes which contained higher sugar content. The cut canes (%) was observed to be significantly low in rodenticides + Tr treated blocks (III and IV, 1-1.35%) as compared to only rodenticide/s treated blocks; significantly minimum yield loss was observed in treated block IV (ZnP+ Br+ Tr). Singla and Parshad (2010) reported 19.12% damaged canes with an estimated 2.67% yield loss. The wheat crop (immature) and potato crop (tuber maturation stage) surrounding sugarcane crop fields perhaps provide a pathway for migration and survival of the rodents providing alternative habitat and food resources. Thus, 2% ZnP followed by 0.005% Br after 15 days interval and 0.25% Tr after further 15 days at cane maturation stage during (December-January) was the best treatment, and triptolide may be effectively used in an IPM of rodents in sugarcane.

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AUTHORS CONTRIBUTION

NK conducted experiments, analyzed data, interpreted results and wrote the manuscript. NS designed research and edited manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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SEASONAL INCIDENCE OF ECTOPARASITIC MITES AND GREATER WAX MOTH IN *APIS MELLIFERA* L COLONIES

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ABSTRACT

The incidence of ectoparasitic mites *Varroa destructor* (Anderson and Trueman), *Tropilaelaps clareae* (Delfinado and Baker) and wax moth *Galleria mellonella* (L.) on the honey bee *Apis mellifera* L. was assessed during 2019-20 at the Krishi Vigyan Kendra, Morena, Madhya Pradesh. The results revealed that *V. destructor* infestation was at minimum (4%) in the first fortnight of July 2019 and maximum (14%) in the first fortnight of September 2019 with the visual examination method. There existed a negative correlation with the brood area. *Tropilaelaps clareae* infestation was minimum (6 mites/ colony) in the second fortnight of November 2019 and maximum (22 mites/ colony) during the first fortnight of October 2019, as assessed by the sugar dusting method. The infestation revealed a negative correlation with colony strength. *Galleria mellonella* incidence was at maximum (176 larvae, 72 pupae, and 17 adults/ colony) in the second fortnight of September 2019 and minimum (5 larvae, 5 pupae, and 2 adults/ colony) in the first fortnight of September, August and August 2019, respectively. The number of pupae and brood area was found significantly negatively correlated (r= -0.534); while the number of adults vs. colony strength and brood area were significantly negatively correlated (r= -0.499 and r= -0.607, respectively).

Key words: *Apis mellifera*, *Tropilaelaps clareae*, *Varroa destructor*, *Galleria mellonella*, seasonal incidence, brood area, colony strength, correlation coefficients, honey store, pollen area

Honey bees are beneficial creatures playing a pivotal role in the pollination of a wide variety of crops and help in maintaining biological diversity (Johannesmeier and Mostert, 2001). These are attacked by many diseases and enemies, which cause weakness of colonies and ultimately low honey production. These include mites, diseases, insect pests, wasps, ants, *Apis dorsata*, lizards, bee-eater birds, etc. Among the ectoparasitic mites, *Varroa destructor* (Anderson and Trueman) is a brood pest of European honey bee *Apis mellifera* L. causing loss of >50% of colonies (Martin et al., 2012; Nazzi et al., 2012). The infestation by *Tropilaelaps clareae* (Delfinado and Baker) causes irregular pattern of sealed and unsealed brood as found with all brood diseases. Modern beekeeping with *A. mellifera* in tropical and subtropical Asia frequently encounters infestation with ectoparasitic mite *T. clareae*. Greater wax moth *Galleria mellonella* L. is the most destructive insect pest of hives and damages the combs causing heavy losses (Williams, 1997; Milam, 1970). Beekeepers should be aware of these enemies and protect their colonies to get the maximum benefits. As only a few attempts have been made to observe the seasonal incidence of parasitic mites and wax moth infesting *A. mellifera*, the present study was conducted.

MATERIALS AND METHODS

The present study was carried out in the apiary at the RVSKVV- Krishi Vigyan Kendra, Morena (26.47°N, 77.98°E, 177 masl), Madhya Pradesh, during 2019-20. Observations were recorded at fortnightly intervals; for colony strength, three randomly selected colonies were observed for the number of frames covered with bees, with both the faces of bee frames taken into account. The brood area and pollen area were recorded in terms of capped brood and pollen comb cells with the help of a wire grid frame (brood measuring frame) by placing it on the hive frame and the number of squares of wire grid covering the brood and pollen area was counted. This provided the brood and pollen area in inch^2 which was converted into cm^2 after multiplying by a factor of 6.45. Honey stores were estimated visually on the assumption that one Langstroth frame sealed with bee wax from both sides of the frame contains 1800-2000 g of honey. The incidence of ectoparasitic mites *V. destructor* and *T. clareae*) in brood and adults was recorded in three selected colonies of *A. mellifera* by

sugar dusting/ visual examination method (Asha et al., 2013; Poonia et al., 2014).

In the visual examination method, 30 brood cells in each selected colony were diagnosed and identified based on their physical appearance with the help of a lens and in the sugar dusting method; 15 g of powdered sugar was kept over the top bar of every bee frame and was gently dusted along with the cells with a bee brush. Mites falling after dusting were collected on the sticky paper screened at the bottom board and were counted after 24 hr. For *G. mellonella* five colonies of equal bee strength were selected randomly and were equalized concerning all colony parameters. All the bees were brushed off the frames and were held against the sunlight and observations were recorded on the number of larvae, pupa, and adults. The experiment was conducted in randomized block design and all the research data were square-root transformed as per the method described by Gomez and Gomez (1986). The data were subjected to statistical analysis by adopting ANOVA as described by Fisher (1958) and by comparing the treatments by using F-test. Data on weather parameters such as temperature, relative humidity, and rainfall was obtained from the Agrometeorological Unit, Zonal Agriculture Research Station, Morena. Correlation coefficients of incidence with weather factors were worked out.

RESULTS AND DISCUSSION

Varroa destructor infestation depicted in Table 1 reveal that it was maximum i.e., 14% in the first Fortnight of September, followed by 11% in the first fortnight of October 2019. The infestation was invariably observed ranging from 4% in the first fortnight of July to 8% in the second fortnight of September 2019. Brood was free from mite infestation in the rest of the months. When it was measured by sugar dusting on top bars of bee frames, a similar trend was seen. The data on the correlation of *V. destructor* incidence with colony and weather parameters showed a significantly negative correlation with brood area {r= -0.522 (visual examination) and r= -0.548 (sugar dusting method)}. During the period of absence of *V. destructor*, it was observed that colony strength and brood area were considerably high. The present results derive support from earlier observations with temperature and rainfall which reported a positive correlation (Deosai and Chhuneja, 2012; Asha et al. 2013; Poonia et al. 2014). Incidence of *V. destructor* was at its peak during September and October corroborating with the results of Fries et al. (1994), Tibor and Szabo (2003) and DeGrandi-Hoffman and Curry (2004).

Seasonal incidence of *Varroa* spp., in *A. mellifera* colonies showed that the maximum infestation occurs during April (12.10± 0.57) in 2008-09; Sharma et al. (2011) observed maximum incidence during November in the Shivalik hills of Himachal Pradesh. Although the peak infestation was noticed in October (72.00± 0.86), November (80.10± 1.58) and December (72.50± 1.27) during 2009-10 (Sharma et al., 2011). Poonia et al. (2014) observed the maximum number of mites in the second fortnight of May (38 and 51 mites/ hive) which showed a significant positive correlation with maximum (r= 0.659) and minimum (r= 0.648) temperature; however, a negative correlation was observed with relative humidity (r= -0.416) and sunshine hours (r= -0.023), whereas rainfall was non-significantly correlated (r= 0.019). It was suggested that during the summer months, when the temperature is high and flower availability is less, the mite population increases. Sharma (2010) while screening *A. mellifera* against *Varroa* reported infestation at Nauni varying from 0.70± 0.15 to 12.10± 0.57. Rinkevich et al. (2017) observed that *V. destructor* can cause devastating losses through direct feeding, vectors for transmitting diseases, and increasing pathogen and parasite susceptibility.

The incidence of *T. clareae* reveals that the brood infestation was noticed initially at 3% in the first fortnight of September 2019. Colonies were invariably infested from 2% in the second fortnight of September to 13% in the first fortnight of November 2019. Colonies were found free from infestation during the remaining months. A similar trend was observed with sugar dusting on top bars of bee frames. The brood infestation was observed to be at minimum of 6 mites/ colony in the second fortnight of November and the maximum was in the first fortnight of October 2019 (22 mites/ colony). Colonies were invariably infested with *T. clareae* from 15 mites/ colony in the first fortnight of September to 19 mites/ colony on the second fortnight of October 2019. The correlation of incidence showed a significant negative correlation with colony strength (r = -0.671) and relative humidity (r= -0.515) by visual examination method; and also showed a significant negative correlation with colony strength (r = -0.552) and brood area (r= -0.591).

The incidence of *T. clareae* in *A. mellifera* had been reported to vary greatly (Camphor and Martin, 2009). The present findings agree with those of earlier workers-Chahal et al. (1986) who observed two peaks period of *T. clareae* infestation i.e. February to May (33.7-51.7%) and September to November (26.8-42.0%), coinciding with a peak of brood rearing activity in Ludhiana, Punjab. Aggarwal and Kapil (1998) reported a high

Table 1. Incidence of *V. destructor*, *T. clareae,* and *G. mellonella* in *A. mellifera* colonies and colony parameters (July 2019- February 2020)

Months	Fort-night	Incidence of *V. destructor*		Incidence of *T. clareae*		Incidence of *G. mellonella*			Colony parameters	
		Visual examination method (%)	Sugar dusting method (no./ colony)	Visual examination (%)	Sugar dusting method (no./ colony)	No. of larvae/ colony	No. of pupae/ colony	No. of adults/ colony	Colony strength (bee frames)	Brood area (cm^2)
July, 2019	1	4.00 (2.12)*	10.00 (3.24)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	4.20	1590.59 (39.89)
	2	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	4.50	1849.51 (43.01)
August	1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	10.00 (3.24)	5.00 (2.35)	2.00 (1.58)	5.20	2042.29 (45.2)
	2	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	27.00 (5.24)	8.00 (2.92)	4.00 (2.12)	5.90	1829.87 (42.78)
September	1	14.00 (3.81)	29.00 (5.43)	3.00 (1.87)	15.00 (3.94)	5.00 (2.35)	37.00 (6.12)	5.00 (2.35)	5.50	1770.67 (42.09)
	2	8.00 (2.92)	19.00 (4.42)	2.00 (1.58)	18.00 (4.3)	176.00 (13.29)	72.00 (8.51)	17.00 (4.18)	5.00	1630.33 (40.38)
October	1	11.00 (3.39)	35.00 (5.96)	9.00 (3.08)	22.00 (4.74)	138.00 (11.77)	56.00 (7.52)	15.00 (3.94)	4.30	1419.17 (37.68)
	2	6.00 (2.55)	18.00 (4.3)	10.00 (3.24)	19.00 (4.42)	87.00 (9.35)	39.00 (6.28)	19.00 (4.42)	3.60	1190.88 (34.52)
November	1	0.00 (0.71)	0.00 (0.71)	13.00 (3.67)	17.00 (4.18)	37.00 (6.12)	13.00 (3.67)	11.00 (3.39)	3.30	1610.21 (40.13)
	2	0.00 (0.71)	0.00 (0.71)	8.00 (2.92)	6.00 (2.55)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	3.70	2361.30 (48.6)
December	1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	4.90	3290.11 (57.36)
	2	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	6.80	3920.49 (62.62)
January, 2020	1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	8.00	4123.63 (64.22)
	2	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	8.40	3409.71 (58.4)
February	1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	7.30	2513.59 (50.14)
	2	5.00 (2.35)	13.00 (3.67)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	6.20	1990.34 (44.62)
CD (p=0.05)		0.12	0.28	0.17	0.29	0.27	0.25	0.37	0.06	0.62

* Figures in parentheses square root √x+0.5 transformed values

infestation rate of *T. clareae* from March to April (21.75-29.80%) and October to November (13.80 &14.23%) at Hisar, Haryana. These findings corroborate with those of Nagaraja and Rajagopal (2003). Thakur et al. (2009) studied seasonal fluctuations in the population of *T. clareae* in *A. mellifera* colonies at Solan, Himachal Pradesh and made observations similar to the present. Padhi and Rath (2012) observed peaks in October followed by November. Sharma et al. (2011) observed peak in colonies during September-October in Himachal Pradesh. Their observations revealed that the *T. clareae* population peaked in September (56.00± 0.31) and October (43.00± 7.40).

The occurrence of *G. mellonella* (larval, pupal as well as adult)/ colony increased from the first fortnight of August till the second fortnight of September 2019; thereafter, started declining till first fortnight of November 2019 (Table 1); maximum larval counts (176 larvae/ colony) was in the second fortnight of September followed by 138 larvae/ colony in the first fortnight of October 2019; while the least of 5 larvae/ colony was in the first fortnight of September 2019. Maximum pupation (72 pupae/ colony) was in the second fortnight of September followed by 56 pupa/ colony in the first fortnight of October 2019, and the least of 5 pupa/ colony was in the first fortnight of August 2019. Maximum adults were observed (19 adults/ colony) in the second fortnight of October followed by 17 adults/ colony in the second fortnight of September 2019, 15 adults/ colony in the first fortnight of October, while the least of 2 adults/ colony was in the first fortnight of August 2019.

Correlation coefficients revealed that the larval counts are negatively correlated with colony and weather parameters, being non-significant. The correlation between the number of pupae and brood area was found significantly negative (r= -0.534). The correlation between the number of adults and colony strength was found negative and significant (r= -0.499) and negative and highly significant for the brood area (r= -0.607). This study on the incidence of larva, pupa, and adult of *G. mellonella* is in line with the other previous studies (Sharma et al., 2011). Raghunandan and Basavarajappa (2014) reported that the colonies with weak populations were more prone to *G. mellonella* infestation and it was more in the semi-arid region during summer (30.80% colonies) followed by the rainy season (23.40%). Kebede et al. (2015) carried out a cross-sectional study from April 28 to May 30, 2009, in four villages of Kafta Humera (Ethiopia) to determine the prevalence of wax moths in modern hive colonies. According to the study, the overall prevalence of *G. mellonella* larvae in modern bee hives was found to be 27.4% and the nature of severity was categorized into three groups. Swati and Verma (2016) studied *A. mellifera* pests in residential and migratory apiaries and its relation with weather parameters; occurrence of *G. mellonela* was significantly higher in migratory apiaries in all the blocks except in Morena and Joura. The incidence of wax moths in all the five blocks except in Morena and Joura was significantly higher (13.6) in migratory apiaries as compared to residential apiaries. The incidence of wax moth larvae was not correlated with the weather parameters except for minimum temperature (r= 0.758).

Sohail et al. (2017) studied the seasonal abundance of *G. mellonella* larvae in hives of honey bees located in district Sargodha, Punjab, Pakistan, and reported that the maximum moth abundance occurred during the regional dearth period, i.e. May-November. The peak abundance (14.8± 3.9 moth larvae/ hive) occurred in August. Usually, weak colonies succumb to moth damage first. The active period of this insect pest has been shown to occur from March to October in India (Garg and Kashyap, 1998) and can remain sustained in November (Brar et al., 1985). In the present study from July 2019 to February 2020, maximum moth abundance occurred from the second fortnight of September 2019 to mid-October, this may be due to increased relative humidity and temperature, unfavorable weather conditions, and scarcity of bee flora (Varshneya et al., 2008). Not surprisingly, wax moth abundance and the % of hives with moth-damaged combs were directly proportional to each other.

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AUTHOR CONTRIBUTION STATEMENT

N. conceived designed research, conducted experiments, and analyzed data. A SY guided and supervised the research. N and N.K. wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EVALUATION OF INSECTICIDES AGAINST SUCKING PESTS OF INDIAN BEAN

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ABSTRACT

Among the various insecticides evaluated for their field efficacy against sucking pests of Indian bean, the treatments of thiamethoxam 25WG at 0.025%, acetamiprid 20SP at 0.004% and buprofezin 25SC at 0.05% were found to be the most effective against *Aphis craccivora* Koch, *Empoasca kerri* Pruthi and *Bemisia tabaci* Gennadius. While, thiacloprid 21.7SC at 0.012% emerged as the moderately effective. Emamectin benzoate 5SG at 0.002%, indoxacarb 14.5SC at 0.007%, lambda-cyhalothrin 5SC at 0.005% and novaluron 10EC at 0.01% were found to be less effective.

Key words: *Lablab purpureus*, *Aphis craccivora*, *Empoasca kerri*, *Bemisia tabaci*, acetamiprid, thiamethoxam, buprofezin, thiacloprid, emamectin benzoate, indoxacarb, lambdacyhalothrin, novaluron

Indian bean *Lablab purpureus* L. is a legume crop widely grown as vegetable or pulse. Insect pests are major constraints in its productivity. It is attacked by a number of insect pests viz*.,* aphid *Aphis craccivora* Koch.; jassids *Empoasca fabae* (Harris)*; E. krameri* Ross and Moore and *E. kerri* Pruthi; pod borer *Etiella zinckenella* (Treit.); whitefly *Bemisia tabaci* (Genn.); stem fly, *Ophiomyia phaseoli* (Tryon); hairy caterpillars *Ascotis imparta (*Walk.*);* Bihar hairy caterpillar, *Spilosoma obliqua* (Walk.) etc. Among these, aphids, jassids and whiteflies are the major sucking pests. These pests attack all parts of the plants including pods which result in stunted growth and decreased yield. The honey dew secretion of the aphids provides a suitable media for the development of sooty mould and fungi which ultimately hamper the process of photosynthesis (David and Kumarswami, 1982). Chemical control of *A. craccivora*, *E. kerri* and *B. tabaci* is usally recommended (Garhwal et al*.,* 1994; Dhamaniya et al*.,* 2005; Yadav et al*.,* 2011) but, due to its continuous and enormous use, problems of resistance, deleterious effect on parasitoids and predators, and residue hazards, and environment pollution have arisen. Hence, there is renewed interest in search for new insecticides, and the present study evaluates some of these.

MATERIALS AND METHODS

The field experiments on the evaluation of efficacy of insecticides were conducted at the College Farm, N M College of Agriculture, Navsari Agricultural University, Navsari, Gujarat during 2019-20. The variety GNIB-22 was used and the seeds sown in plots of size 11 m^2 at 60x 30 cm spacing in 2nd fortnight of October. Eight insecticides viz., thiamethoxam 25WG (1 g/ l), thiacloprid 21.7SC (0.6 ml/ l), buprofezin 25SC (2.0 ml/ l), acetamiprid 20SP (0.2 g/ l), indoxacarb 14.5SC (0.5 ml/ l), emamectin benzoate 5SG (0.4g/ l), lambda-cyhalothrin 5SC (1ml/ l) and novaluron 10EC (1 ml/ l) were evaluated along with untreated control with each replicated thrice. These were applied as a foliar spray on the crop using pre-calibrated knapsack sprayer when the pest incidence was sufficiently built up. Second spray was repeated after 15 days of the first spray. The observations were recorded a day before spray as well as 1st, 3rd, 5th, 7th and 14th days after each spray. The observations were made from five randomly selected plants/ plot. *Aphis craccivora* incidence was observed on three randomly selected twigs (about 10 cm in length). *Empoasca kerri* and *B tabaci* were counted from three leaves (from top, middle and bottom). The pooled data were subjected to statistical analysis.

RESULTS AND DISCUSSION

The pooled results over two sprays given in Tables 1-3 reveal that significantly minimum incidence of *A. craccivora* was observed in plots treated with thiamethoxam 25WG (3.57 aphids/ twig) and it was at par with acetamiprid 20SP (3.82 aphids/ twig) and buprofezin 25SC (4.04 aphids/ twig), while novaluron 10EC (12.49 aphids/ twig) was found less effective (Table 1). Chaudhary et al. (2015) reported that

Table 1. Efficacy of insecticides against *Aphis craccivora* on Indian bean

Tr. No.	Treatments	Before spray	Mean no. of aphids/ twig										Pooled
			First spray					Second spray					
			1DAS	3DAS	5DAS	7DAS	14DAS	1DAS	3DAS	5DAS	7DAS	14DAS	
T_1	Thiamethoxam 25WG at 0.025%	4.05 (16.43)	2.33 (5.44)	2.23 (4.99)	2.05 (4.20)	1.85 (3.42)	2.26 (5.09)	1.94 (3.75)	1.88 (3.52)	1.66 (2.75)	1.39 (1.95)	1.08 (1.16)	1.89 (3.57)
T_2	Thiacloprid 21.7SC at 0.012%	4.13 (17.03)	3.01 (9.04)	2.96 (8.74)	2.84 (8.08)	2.65 (7.00)	2.94 (8.66)	2.68 (7.16)	2.54 (6.48)	2.36 (5.57)	2.16 (4.69)	1.84 (3.38)	2.61 (6.84)
T_3	Buprofezin 25SC at0.05%	3.97 (15.79)	2.41 (5.82)	2.35 (5.54)	2.20 (4.86)	2.02 (4.08)	2.35 (5.52)	2.02 (4.08)	1.91 (3.63)	1.78 (3.19)	1.58 (2.48)	1.25 (1.55)	2.01 (4.04)
T_4	Acetamiprid 20SP at 0.004%	4.12 (16.95)	2.37 (5.62)	2.28 (5.19)	2.13 (4.56)	1.94 (3.79)	2.30 (5.27)	1.98 (3.92)	1.89 (3.57)	1.77 (3.12)	1.54 (2.36)	1.13 (1.27)	1.95 (3.82)
T_5	Indoxacarb 14.5SC at 0.007%	3.95 (15.58)	3.73 (13.89)	3.68 (13.57)	3.59 (12.91)	3.53 (12.46)	3.58 (12.79)	3.36 (11.31)	3.29 (10.84)	3.13 (9.83)	3.00 (8.98)	2.70 (7.30)	3.37 (11.39)
T_6	Emamectin Benzoate 5SG at 0.002%	3.84 (14.72)	3.63 (13.18)	3.59 (12.88)	3.45 (11.94)	3.35 (11.22)	3.52 (12.36)	3.30 (10.87)	3.22 (10.36)	3.08 (9.46)	2.92 (8.50)	2.42 (5.85)	3.26 (10.63)
T_7	Lambda-cyhalothrin 5SC at 0.005%	3.97 (15.76)	3.67 (13.51)	3.63 (13.18)	3.53 (12.46)	3.38 (11.44)	3.54 (12.56)	3.33 (11.08)	3.24 (10.54)	3.10 (9.61)	2.94 (8.66)	2.66 (7.07)	3.31 (11.01)
T_8	Novaluron 10EC at 0.01%	4.05 (16.40)	4.07 (16.56)	3.92 (15.41)	3.82 (14.61)	3.70 (13.73)	3.63 (13.20)	3.43 (11.81)	3.44 (11.85)	3.21 (10.34)	3.09 (9.56)	2.87 (8.23)	3.53 (12.49)
T_9	Control (Treated with water)	4.12 (16.97)	4.65 (21.63)	4.55 (20.67)	4.38 (19.21)	4.22 (17.86)	4.21 (17.76)	3.99 (15.94)	3.94 (15.55)	3.79 (14.38)	3.56 (12.72)	3.27 (10.69)	4.07 (16.56)
	S.E.m ±	0.19	0.18	0.19	0.16	0.14	0.18	0.17	0.14	0.17	0.14	0.13	0.04
	S.E.m ± (P×T)	-	-	-	-	-	-	-	-	-	-	-	0.06
	C.D (p= 0.05)	NS	0.55	0.57	0.48	0.44	0.55	0.51	0.41	0.52	0.43	0.38	0.13
	C.D (p= 0.05) (P×T)	-		-	-	-	-		-	-	-	-	NS

DAS = Days after spraying; Figure in parentheses original, and outside $\sqrt{x + 0.5}$ transformed values.

Table 2. Efficacy of insecticides against *Empoasca kerri* on Indian bean

Tr. No.	Treatments	Before spray	Mean no. of jassids/ leaf										Pooled
			First spray					Second spray					
			1DAS	3DAS	5DAS	7DAS	14DAS	1DAS	3DAS	5DAS	7DAS	14DAS	
T_1	Thiamethoxam 25WG at 0.025%	2.93 (8.58)	1.91 (3.65)	1.95 (3.82)	1.88 (3.55)	1.73 (2.98)	1.92 (3.67)	1.68 (2.83)	1.73 (2.99)	1.56 (2.42)	1.17 (1.36)	0.95 (0.90)	1.67 (2.80)
T_2	Thiacloprid 21.7SC at 0.012%	2.91 (8.47)	2.32 (5.37)	2.37 (5.63)	2.31 (5.35)	2.22 (4.94)	2.31 (5.35)	2.17 (4.71)	2.19 (4.82)	2.04 (4.15)	1.79 (3.22)	1.49 (2.22)	2.14 (4.60)
T_3	Buprofezin 25SC at0.05%	2.86 (8.16)	2.30 (5.29)	2.34 (5.47)	2.29 (5.28)	2.20 (4.86)	2.29 (5.25)	2.13 (4.55)	2.17 (4.72)	1.95 (3.82)	1.72 (2.96)	1.38 (1.90)	2.10 (4.41)
T_4	Acetamiprid 20SP at 0.004%	2.83 (7.99)	1.89 (3.56)	1.91 (3.67)	1.84 (3.39)	1.71 (2.92)	1.88 (3.56)	1.66 (2.76)	1.69 (2.85)	1.51 (2.29)	1.10 (1.22)	0.88 (0.77)	1.63 (2.67)
T_5	Indoxacarb 14.5SC at 0.007%	2.88 (8.29)	2.74 (7.51)	2.78 (7.74)	2.74 (7.54)	2.70 (7.27)	2.74 (7.52)	2.63 (6.92)	2.67 (7.12)	2.52 (6.35)	2.31 (5.34)	2.09 (4.38)	2.61 (6.82)
T_6	Emamectin Benzoate 5SG at 0.002%	2.90 (8.39)	2.71 (7.36)	2.74 (7.54)	2.71 (7.34)	2.66 (7.06)	2.72 (7.41)	2.60 (6.78)	2.65 (7.02)	2.47 (6.08)	2.30 (5.29)	2.09 (4.35)	2.58 (6.67)
T_7	Lambda-cyhalothrin 5SC at 0.005%	2.82 (7.95)	2.77 (7.65)	2.80 (7.87)	2.75 (7.59)	2.71 (7.34)	2.78 (7.73)	2.65 (7.04)	2.71 (7.34)	2.56 (6.55)	2.36 (5.59)	2.15 (4.64)	2.64 (6.99)
T_8	Novaluron 10EC at 0.01%	2.87 (8.23)	2.80 (7.86)	2.83 (8.00)	2.78 (7.75)	2.75 (7.56)	2.80 (7.88)	2.69 (7.23)	2.73 (7.49)	2.56 (6.55)	2.41 (5.81)	2.19 (4.80)	2.67 (7.15)
T_9	Control (Treated with water)	2.84 (8.10)	2.99 (8.96)	3.03 (9.16)	2.99 (8.92)	2.94 (8.64)	2.99 (8.98)	2.84 (8.08)	2.88 (8.34)	2.86 (8.20)	2.69 (7.25)	2.53 (6.40)	2.89 (8.36)
	S.E.m ±	0.12	0.13	0.11	0.12	0.14	0.12	0.14	0.12	0.09	0.11	0.10	0.03
	S.E.m ± (P×T)	-	-	-	-	-	-	-	-	-	-	-	0.05
	C.D (p= 0.05)	NS	0.39	0.34	0.36	0.40	0.37	0.41	0.36	0.27	0.34	0.32	0.09
	C.D (p= 0.05) (P×T)	-		-	-	-	-		-	-	-	-	NS

DAS = Days after spraying; Figure in parentheses are original value whereas, those outside are $\sqrt{x} + 0.5$ transformed values.

Table 3. Efficacy of insecticides against *Bemisia tabaci* on Indian bean

Tr. No.	Treatments	Before spray	Mean no. of whitefly/ leaf										Pooled
			First spray					Second spray					
			1DAS	3DAS	5DAS	7DAS	14DAS	1DAS	3DAS	5DAS	7DAS	14DAS	
T_1	Thiamethoxam 25WG at 0.025%	2.73 (7.45)	2.11 (4.47)	2.12 (4.48)	1.84 (3.39)	1.63 (2.68)	1.86 (3.46)	1.47 (2.16)	1.48 (2.18)	1.39 (1.92)	1.18 (1.39)	1.01 (1.03)	1.63 (2.68)
T_2	Thiacloprid 21.7SC at 0.012%	2.68 (7.18)	2.53 (6.40)	2.56 (6.55)	2.31 (5.35)	2.13 (4.54)	2.30 (5.27)	1.99 (3.97)	2.00 (4.01)	1.92 (3.67)	1.74 (3.03)	1.54 (2.36)	2.12 (4.51)
T_3	Buprofezin 25SC at0.05%	2.75 (7.54)	2.14 (4.59)	2.14 (4.58)	1.86 (3.47)	1.70 (2.89)	1.90 (3.62)	1.50 (2.24)	1.52 (2.30)	1.45 (2.10)	1.22 (1.48)	1.07 (1.15)	1.67 (2.82)
T_4	Acetamiprid 20SP at 0.004%	2.71 (7.36)	2.09 (4.38)	2.10 (4.41)	1.83 (3.36)	1.62 (2.62)	1.83 (3.36)	1.40 (1.96)	1.43 (2.04)	1.33 (1.77)	1.16 (1.34)	0.94 (0.90)	1.60 (2.57)
T_5	Indoxacarb 14.5SC at 0.007%	2.82 (7.97)	2.73 (7.43)	2.79 (7.80)	2.65 (7.05)	2.54 (6.47)	2.63 (6.93)	2.40 (5.76)	2.43 (5.92)	2.32 (5.37)	2.11 (4.47)	1.92 (3.67)	2.47 (6.11)
T_6	Emamectin Benzoate 5SG at 0.002%	2.76 (7.64)	2.72 (7.38)	2.77 (7.69)	2.63 (6.93)	2.53 (6.40)	2.62 (6.88)	2.38 (5.66)	2.42 (5.84)	2.32 (5.37)	2.10 (4.42)	1.89 (3.56)	2.45 (6.03)
T_7	Lambda-cyhalothrin 5SC at 0.005%	2.80 (7.84)	2.76 (7.60)	2.81 (7.93)	2.67 (7.12)	2.58 (6.67)	2.64 (7.00)	2.45 (6.03)	2.51 (6.28)	2.35 (5.54)	2.16 (4.65)	2.00 (4.00)	2.51 (6.31)
T_8	Novaluron 10EC at 0.01%	2.70 (7.30)	2.76 (7.65)	2.83 (8.00)	2.72 (7.41)	2.61 (6.83)	2.68 (7.16)	2.47 (6.11)	2.52 (6.37)	2.37 (5.62)	2.21 (4.87)	2.05 (4.22)	2.54 (6.46)
T_9	Control (Treated with water)	2.79 (7.78)	2.90 (8.45)	3.01 (9.08)	3.04 (9.24)	2.96 (8.47)	3.00 (9.00)	2.85 (8.16)	2.89 (8.39)	2.82 (7.95)	2.71 (7.34)	2.46 (6.04)	2.88 (8.31)
	S.E.m ±	0.13	0.13	0.12	0.10	0.10	0.10	0.12	0.12	0.13	0.10	0.08	0.06
	S.E.m ± (P×T)	-	-	-	-	-	-	-	-	-	-	-	0.04
	C.D (p= 0.05)	NS	0.38	0.36	0.32	0.32	0.30	0.37	0.37	0.38	0.31	0.25	0.20
	C.D at 5 % (P×T)	-	-	-	-		-	-	-	-		-	0.12

DAS = Days after spraying; Figure in parentheses are original value whereas, those outside are $\sqrt{x} + 0.5$ transformed values.

imidacloprid followed by acetamiprid were superior; Choudhary et al. (2017) showed that thiamethoxam (0.005%) was the most effective. As regards the incidence of *E. kerri*, acetamiprid 20SP (2.67 jassids/ leaf) followed by thiamethoxam 25WG (2.80 jassids/ leaf) were the best (Table 2); buprofezin 25SC and thiacloprid 21.7SC were the next best. Chaudhary et al. (2015) found that imidacloprid followed by acetamiprid were superior; while Singh et al. (2019) showed that acetamiprid (0.004%) was the most effective in green gram. Meena et al. (2020) reported that imidacloprid > thiamethoxam > acetamiprid were the most effective in green gram. The incidence of *B. tabaci* was significantly reduced with acetamiprid 20SP (2.57 whiteflies/ leaf) and it was at par with thiamethoxam 25WG (2.68 whiteflies/ leaf); it was followed by buprofezin 25SC and thiacloprid, while was the least effective (Table 3). These results agree with those of Chaudhary et al. (2015) that incidence of whitefly was significantly reduced with acetamiprid. Kukvaya et al. (2018) revealed that thiamethoxam 25 WG @ 0.005 was highly effective against whitefly in moth bean. Singh and Singh (2018, 2019) observed that acetamiprid was the most effective in green gram.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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DIVERSITY OF BUTTERFLY POLLINATORS IN AGROECOSYSTEMS OF KUMAUN REGION

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ABSTRACT

This study examined the diversity, abundance and evenness of butterfly pollinators in Kumaun region, Uttarakhand from March 2019 to February 2020. A total of 394 butterflies, representing 40 species and five families were collected. Nymphalidae was the most dominant family with 19 species, followed by Pieridae (9 species), Lycaenidae (9 species), Papilionidae (2 species), and Hesperiidae (1 species), respectively. *Pieris canidia indica* (Evans) was the most abundant species, constituting 38.3% of the total individuals. Shannon-Wiener diversity index was observed to be maximum in Site-1 (H'=1.37) than in Site-2 (H'=1.12). Similarly, evenness was maximum in Site-1 (E=0.457), than in Site-2 (E=0.333). In addition, significant pollinator species in crops have been identified in agroecosystems.

Key words: Lepidoptera, agroecosystem, species richness, evenness, distribution, abundance, pollination, species composition, diversity indices, *Pieris canidia indica*

Insects are the most dominating and diverse organisms on earth, inhabit all habitat types and play major roles in the function and stability of terrestrial and aquatic ecosystems (Godfray, 2002). Slightly over one million of insect species have been described, of which five insect orders stand out to be the most abundant in their levels of species richness: Hymenoptera, Diptera, Coleoptera, Lepidoptera, and the Hemiptera (Jach and Balke, 2008). The Lepidoptera with >1,50,000 species is the second largest and the most diverse (Risley, 1986; Gullan and Cranston, 2010). Butterflies are generally colorful winged insects with an overlay of tiny scales, and thus, butterflies are the planet's most majestic creatures with brilliantly colored and exquisitely patterned (Mayur et al., 2013). They have always been fascinating (Kehimkar, 2008). Butterflies are one of the best taxonomically studied groups of insects (Pollard, 1991; Robbins et al., 1997; Kunte, 2000). Pollinators play an immense role in producing many fruits, vegetables and field crops (Klein et al., 2007). Pollination not only improves the yield of the crop but also contributes to uniform and early pod setting (Abrol, 2007) which increase production (Aizen et al., 2008). Hymenoptera, Diptera, Coleoptera, Lepidoptera, Thysanoptera, Hemiptera and Neuroptera are of prime significance in the crop pollination (Free,1993; Kearns et al.,1998; Bhowmik et al., 2014). Butterflies also play an important role in pollination (Patil et al., 2017; Martínez-Adriano et al., 2018). The relationship between flowering plants and flower-visiting insects is essential for conserving the terrestrial ecosystem and leading to different interactions between insect-plant and insect-herbivore (Soler et al., 2009). Tiple (2011) reported a total of 1504 lepidopterans from the Indian Subcontinent. There are only a few studies reported on diversity of butterflies in Kumaun region (Tyagi et al., 2011; Gariya 2016; Arya 2020). The present study aims at finding out the species composition, distribution and status of butterflies in the agroecosystems of Paharpani and Betalghat, Nainital, Kumaun region, Uttarakhand.

MATERIALS AND METHODS

The present study was conducted in agroecosystems located at Paharpani (2044 masl, 29° 25'38N, 79° 42'41E), Betalghat (774 masl, 29° 33'20N, 79° 20'51E) in Nainital district of Uttarakhand from March 2019 to February 2020. In Paharpani, (*Brassica oleracea*) L., (*Brassica sinapis*) L., (*Coriandrum sativum*) L., (*Solanum tuberosum*) L., (*Cucumis sativus*) L., (*Pisum sativum*) L., (*Allium cepa*) L., (*Raphanus sativus*) L., (*Phaseolus vulgaris*) L., (*Capsicum annuum*) L., (*Phaseolus vulgaris*) L., (*B. oleracea*) L. are grown. In Betalghat, (*S. tuberosum*) L., (*Brassica sinapis*) L., (*P. sativum*) L., (*Abelmoschuc esculentus*) L., (*A. cepa*) L., (*Coriandrum sativum*) L., (*B. oleracea*) Var. batrytis., (*Cucurbita pepo*) L., (*S. melongena*) L., (*Solanum lycopersicum*) L. are grown. Sites were visited regularly on monthly basis between 9:00 am to 2:00 pm, when the butterflies were most active. Sampling of butterflies was done at an interval of 30 days. The butterflies were collected by sweep method and hand picking. The sweep net with a diameter of 30 cm at the mouth

Table 1. Species of butterflies (Paharpani and Betalghat, Kumaun region, Nainital, Uttarakhand.

S. No.	Species	Common name	Site-1	Site-2	Total
		Family: Pieridae			
1.	*Pieries canidia indica* (E)	Indian cabbage white	15	30	45
2.	*Pontia daplidice* (L)	Bath white	8	12	20
3.	*Eurema brigitta rubella* W	Small grass yellow	-	28	28
4.	*Eurema laeta* B	Spotless grass yellow	-	4	4
5.	*Goneptyryx rhamni nepalensis* D	Common brimstone	8	4	12
6.	*Aporia agathon* (G)	Great blackvein	6	5	11
7.	*Colias felidi* M	Dark clouded yellow	13	7	20
8.	*Catopsilia pyranthe* L	Mottled emigrant	-	7	7
9.	*Pareronia hippia* F	Indian wanderer	-	4	4
		Family: Nymphalidae			
10.	*Vanessa indica* H	Indian red admiral	-	4	4
11.	*Vanessa cardui* L	Painted lady	-	3	3
12.	*Aglais caschmirensis* K	Indian tortoiseshell	12	18	30
13.	*Junonia lemmonias* L.	Lemon pansy	-	7	7
14.	*Junonia ipitha* C	Chocolate pansy	-	2	2
15.	*Neptis hylas* L	Common sailor	-	4	4
16.	*Ypthima inica* H	Lesser three ring	5	-	5
17.	*Ypthima baldus* F	Common five ring	-	3	3
18.	*Callerebia scanada* K	Pallid argus	8	2	10
19.	*Callerebia nirmala* M	Common argus	5	-	5
20.	*Collerebia hybrida* B	Hybrid argus	-	4	4
21.	*Acraea issoria* H	Yellow coster	7	-	7
22.	*Ariadne merione* C	Common castor	-	4	4
23.	*Hypolimnas bolina* (L)	Great eggfly	-	1	1
24.	*Lasiommata schakra* K	Common wall	8	-	8
25.	*Euthalia patala* K	Common baron	-	3	3
26.	*Lethe rohria* F	Common treebrown	-	2	2
27.	*Danaus chrysippus* L	Plain tiger	-	6	6
28.	*Parantica aglea* S	Glassy tiger	-	2	2
		Family: Lycaenidae			
29.	*Lycaena pavana* K	White bordered copper	24	-	24
30.	*Lycaena phlaeas* L	Common copper	-	3	3
31.	*Euchrysops cnejus* F	Gram blue	4	-	4
32.	*Heliophorus moorei* (H)	Azure sapphire	9	-	9
33.	*Dodona durga* K	Common punch	8	-	8
34.	*Talicada nyseus* (G)	Red pierrot	-	6	6
35.	*Zizeeria sp.*		20	12	32
36.	*Pseudozizeeria maha* K	Pale grass blue	9	-	9
37.	*Zizina otis* F	Lesser grass blue	10	-	10
		Family: Papilionidae			
38.	*Papilio demoleus* L	Lime butterfly	-	2	2
39.	*Papilio polytes* L	Common Mormon	8	12	20
		Family: Hespiriidae			
40.	*Borbo bevani* M	Bevan's swift	6	-	6
	Total		193	201	394

and the bag length of 60 cm was used. The collected butterflies were killed with ethyl acetate soaked cotton and brought to the laboratory, processed and stretched in wooden boxes. The collected specimens were identified through various standard literatures. (Kumar, 2008; Singh, 2011). Biodiversity indices were calculated following Shannon-Wiener (1963) and evenness index (Hill, 1973).

RESULTS AND DISCUSSION

During the present study, a total of 40 species of butterflies belonging to 5 families were collected (Table 1; Figs. 1-9). Out of these, nine species each belonged to the families Pieridae and Lycaenidae, 19 species to Nymphalidae, two species to Papilionidae and one species to family Hesperiidae. A total of 20 species were collected from site-1 and 29 species from site-2. Maximum species belonged to Pieridae (38.3%) and Nymphalidae (27.9%). A total of 394 individuals of butterflies were collected in two study sites: 193 individuals belonged from site-1 and 201 individuals from site-2. In terms of number of species, family Nymphalidae was the most dominant with 19 species (47.5%) followed by Pieridae and Lycaenidae (9 species, 22.5%) in each, Papilionidae (2 species, 5%) and Hesperiidae (1 species, 2.5%). Relative abundance (%) of individuals of different families are shown in Fig. 1. Overall, maximum number of individuals belonged to the Pieridae (151 individuals, 38.3%).

Temperate region of Nainital, Kumaun region, Uttarakhand commonly consists of different crops and vegetables. Different workers have documented the study of butterflies in different parts of the Nainital district (Tyagi et al., 2011; Kapkoti et al., 2016; Arya et al., 2017; Meena et al., 2017; Sanwal et al., 2017). The present study showed that the pollinators diversity was more in Paharpani, having more varied vegetation type and floral vegetation in comparison to Betalghat. Our findings clearly indicated that all 40 species of butterflies collected were the most diverse pollinators in the agro-ecosystem studied. The large body of butterflies helps in sticking of pollens to their legs and proboscis when they visit another flower thus making butterflies a very effective pollinator of crops (Duara and Kalita, 2013; Rader et al., 2016; Das et al.,2018). Diversity indices provide important information about rarity and commonness of species bin a community. Shannon index of diversity is considered to be the most complete measures of diversity because it takes into account both number of species and the abundance of each species. Shannon-Wiener's diversity index was higher in site-1 (H′=1.37), than in site-2 (H′=1.12). Similarly, Evenness was higher in site-1 (E=0.457), than in site-2 (E=0.333). The results obtained for diversity indices in the present study indicate that values obtained are comparable to other reported values for Lepidoptera in different agro-ecosystems (Arya et al., 2014, Usha and John, 2015).

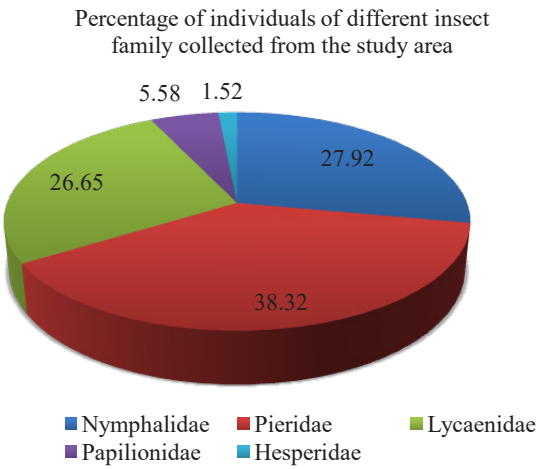


Fig. 1. Relative abundance (%)- families

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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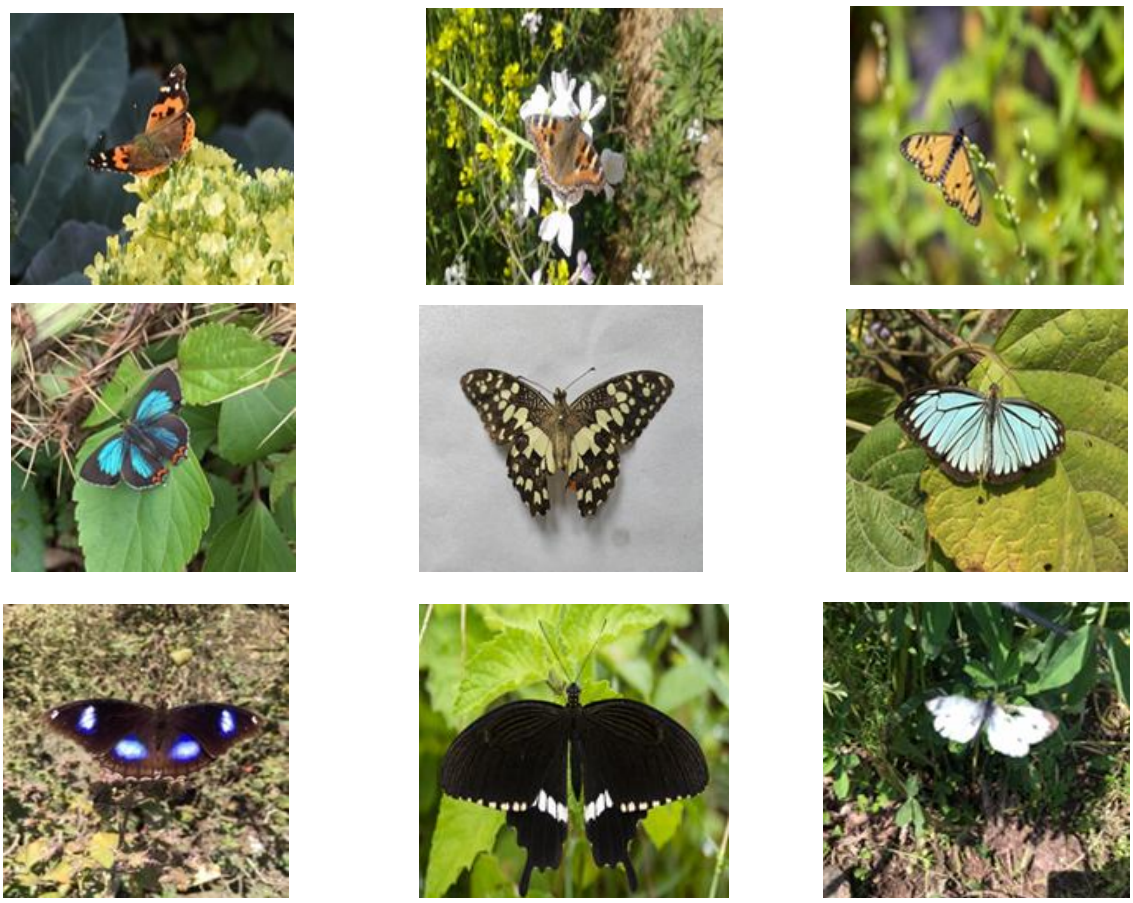


Fig. 2-10. Habitus of abundant butterflies: 1. Vanesaa indica; 2. Aglais cashmirensis; 3. Acraea issoria; 4. Heliophorus moorei; 5. Papilio demolus; 6. Pareronia hippia; 7. Hypolimnas bolina; 8. Papilio polytes; 9. Pieris canidia indica

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THERAPEUTIC USE OF INSECTS AND INSECT PRODUCTS

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ABSTRACT

Insects and their products have been used as resources of medicines all over the world since ancient times. Insect-derived compounds for therapeutic use have huge promise as they have remedial, analgesic, antibacterial, diuretic, sedative, anticancer and anti-rheumatic properties. In old traditional medicine, insect products such as honey, venom, maggots, silk and cantharidine etc. have been used as a treatment for many human ailments. Therapies based on insects are now finding increased acceptance and importance because of failure of substitute drug delivery methods to deliver many lead compounds in key therapeutic areas. There are a number of new advancements in the development of insect natural compounds as prospective new medicines. This review provides updated information regarding traditional and modern use of insects as medicines across various parts of the world.

Key words: Analgesic, diuretic, anaesthetic, cantharidine, entomotherapy, immunological, therapeutic, maggots, traditional, venom, apitherapy, silk, AMPs, insects models, commercial products

Insects belong to phylum Arthropoda which is the largest phylum of animal kingdom as it comprises more than 80% of all known animal species. Among all these species, insects are one of the most diverse and successful groups of animals in the whole world. As a result of this vast diversity, resources obtained from insects are also very large and diversified (Leonhardt et al., 2020). Insects produce a wide range of biologically active low and high molecular compounds mostly as defence mechanisms. Insect based natural molecules are diverse in structure and function ranging from fatty acids, polyketide, terpenoids, nucleosides etc (Rather et al., 2020). When these compounds (venoms, toxins) are consumed by humans, they may act as drugs as they have medicinal effects or improve the performance (Bordon et al., 2020).

Insects are an excellent source of many valuable natural products. The natural products obtained from insects have marvellous pharmacological potential in comparison to another group of animals (Kumar and Srivastava, 2020). Insects have consistently helped humanity by offering a variety of services. People have been using insects in many ways to make natural products since ancient times (Matthäus et al., 2019). The most commonly used insects were honey bees for products such as honey and wax. Also, the products obtained from apiculture have been used long ago in diet and for phytotherapy due to their positive health effects (Gabriele et al., 2015). Scale insects have been used for production of lac dye, resin, and wax long ago. The history of using lac insects can be traced back to Vedic period (Sharma et al., 2020). Silkworms and their by-products have been used since traditional times due to their remarkable therapeutic properties and other usages. Other than production of silk, silkworms have been utilized as nutrients for human consumption or as cattle feed (Bashir et al., 2022). In indigenous societies with little 'westernization', traditional ethnobiological knowledge and consumption of insects is still practiced. The knowledge about therapeutic uses of insects are a well kept secret and passed from generation to generation (Chakravorty et al., 2011).

The medicinal use of insects and insect-derived products is known as entomotherapy (Costa-Neto, 2005). Insects and the substances derived from them have long been used as medicinal resources in the medical systems. In many social cultures, human beings have exploited animal resources for medicinal reasons since the ancient time, where traditional cures were made from animal body parts or metabolic by products or non-animal materials (Unnikrishnan, 1998). Due to the imbalanced patient-to-healthcare professional ratios, the increased workload for the latter, various side effects of modern medicine, the inability to completely cure chronic diseases, the high cost of new medications, and the emergence of new diseases, alternative medicine

has recently attracted renewed public interest (Akter et al., 2021). Insects have shown to be extremely important as sources of modern pharmaceuticals due to their immunological, analgesic, antibacterial, diuretic, anaesthetic and antirheumatic properties (Yamakawa, 1998). In the present times, insect natural secretions have demonstrated wide potential as alternative medicine and many insect resources have been utilized and industrialized for various medicinal therapies (Zhang et al., 2008). Products made from insects have an annual economic value of about $100 million (Kasozi et al., 2020). Therefore, insects offer an endless source of potential therapeutic substances. This paper is reviewed to explore therapeutic utilization of insect resources in ancient and modern times in India and other parts of the world.

Traditional entomotherapy

Around the world

Insects have produced valuable natural products like bee-wax, propolis, pollen and royal jelly etc, which have been utilised in traditional medicine for a long time (Jamasb et al., 2016). Many segments of society continue to use medico-entomological medications to this day in many regions of the world. The identification of biological resources suitable for commercial exploitation has been significantly aided by the traditional medical knowledge of regional cultures all over the world (Feng, 2009). The knowledge and methods about the therapeutic use of insects have been passed down through the generations and they are effectively incorporated into the surrounding culture (Costa-Neto, 2005). In recent years, a lot of studies has been conducted which draws the attention towards the therapeutic value of various species of insects, their products and their developmental stages since the ancient times (Chakravorty et al., 2011). A few examples of traditional use of insects as medicines around the world are explained as:

Maggots have been used for wound healing in folk medicines by the Aborigines and Mayan for thousands of years (Pemberton and Robert, 1999). During Napoleonic and American civil wars, maggots were used for wound cleaning (Ratcliffe et al., 2011). In Iran, Kerman province was endemic area for leishmaniasis and for treating cutaneous leishmaniasis meloid beetles (*Leishmania tropica*) were used as traditional medicine (Nikbakhtzadeh and Tirgari, 2002). In Brazil, tenebrionid beetle (*Palembus dermestoides*) were used to treat asthma, arthritis, tuberculosis and sexual impotence (Costa-Neto, 2005). Amazonian Indians diagnosed diabetes by watching to see whether ants swarmed over urine which in diabetics contains high levels of sugar to attract the ant (Lockhart, 2007).

In various areas of Southeast Asia, as well as all of East Asia, insect is a typical component of contemporary medical care. There are 77 distinct species, 14 families, and 8 orders of insects which have traditionally been used in China to treat cancer and tumours (Jiang, 1990). In Chinese traditional medicine, extracts of insect corpses have been widely utilised to treat a wide range of ailments including flu, cancer and infections of throat and ear (Pemberton and Robert, 1999; Costa-Neto, 2005). The use of Chinese Black Mountain Ant, *Polyrhachis vicina*, is a wonderful example, that is frequently used as a remedy especially by the Chinese elder persons. It is intended to increase virility and fertility, lengthen life, profit from anti-aging, and prolong life. British experts are looking into the extract's potential as a cancer-fighting substance in light of the new interest in the ants' medicinal properties (Chen, 1994). Silkworms (*Bombyx mori* L. 1758) provides another popular example which have been used in Chinese traditional medicine for at least three thousand years and specific fly larvae of species *Lucilia* have been known to be effective healers for infected wounds for generations (Sherman, 2009).

Traditional insect medicine in Africa is highly diverse in comparison to China and India (Srivastava et al., 2009). Unlike Traditional Chinese Medicine and Ayurveda, most bug medicinal remedies are passed down through families and communities as opposed to being taught in universities (Patwardhan et al., 2005). In Africa, grasshoppers are frequently consumed as a delicacy as well as a good source of protein and for medicinal purposes. These insects are typically collected, sun-dried, and then ground into a powder. This powder can then be combined with water or ash to make a paste that can be applied to the forehead to reduce severe headaches (Hill and Goddard, 2012). Fasoranti (1997) explained that mole crickets (*Gryllotalpa africana*) gut contents are applied to infected feet as a treatment in southwest Nigeria.

Since ancient times, the therapeutic powers of several insect species and a wide array of plant species have been recognised in Mexico. In some local markets, insect parts are sold in markets as diuretics, analgesics, anaesthetics and aphrodisiacs. About 43 bug species have been found to be utilised in traditional medicine

as ointments, pomades, or infusions (Conconi and Moreno, 1988). Additionally, they have been produced and utilised in a variety of methods to treat a wide range of illnesses, including urogenital immunological and glandular disorders, stomach pain, liver issues and mental breakdowns (Taylor, 1975).

In India

In India, Ayurveda is an ancient and integral part in traditional medical therapy that is almost always utilised in conjunction with Western medicine (Pandey et al., 2013). In the Sanskrit Veda of ancient India, it has been mentioned that honey and honey bee products have healing properties and was a remedy for many disorders (Gupta and Stangaciu, 2014). In ayurvedic scriptures, honey is called madhu and was one of the most important medicines used in ayurvedic period (Liyanage and Mawatha, 2017). Termites were believed to treat a broad variety of illnesses, both specific and general during Ayurvedic era. The termites and the elements of their architectural mounds are ground into a paste that is applied topically to the affected areas or, less frequently, mixed with water and consumed. According to reports, this drug is beneficial in treating anaemia, rheumatic diseases and ulcers and used as a general pain reliever and health enhancer (Figueirêdo et al., 2015). In Atharva Veda, it has been mentioned that lac extract was widely used on open wounds for healing and tissue regeneration (Perveen et al., 2013).

In Ayurveda, another example of therapeutic use of insect was of jatropha leaf miner. It is a lepidopteran which is a major agricultural pest of jatropha. It was believed that topical application of its larvae induce lactation, reduce fever, and soothe gastrointestinal tracts (Srivastava et al., 2009). Even today, traditional knowledge about entomotherepy has been practiced among the ethnic societies of North-East India. This traditional knowledge is a well kept secret and is only transferred from one generation to another (Chakravorty et al., 2011). Earlier several workers described therapeutic use of insects based on traditional knowledge in several tribes of India. Some of the important findings are described in Table 1.

Modern entomotherapy

Since the revolutionary invention of antibiotics, relatively little study has been done in medical entomology, despite the fact that insects have been utilised in medicinal therapy extensively throughout history for almost on every continent (Dossey, 2010). But due to enhancement of antibiotic-resistant infections, pharmaceutical sector explored new resources other than antibiotics (Lojewska and Sakowicz, 2021).

Table 1. Insect orders having therapeutic potential and their primary use in traditional medicine in India

Sl. No.	State	Common name	Scientific name	Insect order	Disease treated	Reference
1.	Assam	Blister beetle	*Melolontha* spp.	Meloidae	Cancer	Verma and Prasad, 2013
2.	West Bengal	Stingless bee	*Lepidotrigona arciferal*	Hymenoptera	Cold and cough, cancer	Biswa et al., 2017
3.	Tamil Nadu	Honey bee	*Apis indica*	Hymenoptera	Used as eye drops	Solovan et al., 2004
4.	Chhattisgarh	Kambal keeda	*Diacrisia oblique*	Lepidoptera	Dog bite	Srivastava et al., 2009
5.	Chhattisgarh	Pod borer	*Helicoverpa armigera*	Lepidoptera	Fever, nervous breakdown, eosinophilia and asthma	Oudhia, 2001
6.	Orissa	Termite spp.	-	Isoptera	Wound healing in cattle	Paul and Sudipta, 2011
7.	Manipur	Cranefly	*Tipula* spp.	Diptera	Healing process and to relieve body aches	Kapesa et al., 2020
8.	Arunachal pradesh	Red tree ant	*Oecophylla smaragdina*	Hymenoptera	Stomachache and Dysentery	Chakravorty et al., 2011
9.	Nagaland	Treehopper	*Darthula hardwickii*	Hemiptera	Body ache, jaundice	Mozhui et al., 2020
10.	Nagaland	Litchi stink bug	*Tessaratoma javanica*	Hemiptera	Warts removal	Pongener et al., 2019

Arthropods are a valuable and mostly untapped source of novel pharmaceutical chemicals (Ebenebe et al., 2021). The secretions produced by these arthropods acts as a defence mechanism. It includes insect venom from honeybees and wasps, blood-feeding insects like blister beetles and maggots, fruit flies, antimicrobial peptides of bee hives etc (Rádis-Baptista and Konno, 2020). Scientists have explored that venoms or toxins produced by arthropods included many inorganic or organic compounds, alkaloids, histamines, enzymes etc (Lee and Bae, 2016). A number of insect based compounds having therapeutic values are developed nowadays which are described as under:

Apitherapy

Apitherapy is the use of bee products such as honey, pollen, propolis, royal jelly, bee venom and wax to treat gastrointestinal, cardiovascular and hepatic diseases as well as to hasten the healing of wounds (Abdulrhman et al., 2010). More than 6000 years ago, ancient Egyptian medicine was the origin of apitherapy. Honey is a naturally sweet material made by honeybees from the nectar of blossoms, plant secretions, or excretions of insects that feed on living plants (Bansal et al., 2005). Honey bees collect these ingredients, transform and combine them with other ingredients, then store and let them develop in the honey comb. There are numerous health benefits of honey due to its antibacterial, wound-healing, antidepressant, anticonvulsant, and anti-anxiety properties (Simon et al., 2005).

Bees use pollen as a basic ingredient to make bee bread. Bee pollen has a complex chemical composition that includes carbohydrates, proteins, amino acids, vitamins and minerals (Silva et al., 2014). It is a highly nutritious food that is good for health because it contains phenolic compounds that have antioxidant activity. Since a long time, bee pollen has been used as an anti-aging and anti-allergic remedy (Basa et al., 2016). Honey bees collect propolis, sometimes known as "bee glue," a resinous honeybee product from a number of plant sources. It has traditionally been used in medicines, for the last many centuries to treat burns, ulcers, bedsores, other wounds difficult to heal and as a mouth disinfectant (Kędzia and Kędzia, 2013). Additionally, a number of biological effects of bee propolis, including anticancer, antioxidant, antibiotic, antifungal and anti-hepatotoxic characteristics, have been reported (Lee and Bae, 2016). Like other honey bee products, royal jelly is nutrient-dense and has a high nutritional value. It has been utilised to increase vigour in the body and fortify the immune system (Adjare, 1990). Bee venom is secreted from the glands connected to sting apparatus in worker bees. In bee venom therapy, live bee stings are used to treat a number of ailment symptoms such as discomfort, lack of coordination and muscle weakness (Uddin et al., 2016). Bee venom has been used to treat a number of inflammatory conditions due to its anti-inflammatory properties, in various ailments like arthritis, bursitis, tendinitis, keloids, herpes, zoster, joint disease and rheumatoid arthritis (Adjare, 1990). Experts in apitherapy hold that some chemicals in bee venom, include melittin, apamin, phospholipase A and adolapin, can lessen inflammation and pain, and that the mixture of all the components in bee venom helps the body to produce natural healing molecules in its own defence (Zhang et al., 2018).

Silk

In order to be used outside of the body, silk is a structured protein that is spun into a fibre in number of insects (Becker et al., 2003). Silks are self-assembling, biodegradable proteins that can be genetically modified to have certain chemical properties, making them suitable for the transport of genes and medications (Xiao et al., 2014). Silk has been long used in biomedical sutures for long time, due to which silkworm silk has been given approval by FDA for its extended biomaterials device usage (Zhou et al., 2017). Due to the diversity and control of size, shape and chemistry, modified or recombinant silk proteins can be created and employed in a number of biomedical applications, including the transportation of bioactive substances (Numata et al., 2009). Research on silk films has focused on tissue engineering scaffolds that support cells, devices for controlled substance (like drug) release and biochemical sensors. The majority of these studies have utilised silk fibroins from the silkworm *B. mori*, although recombinant spider silk proteins may be utilised in a similar manner due to their similar physico-chemical properties (Kim et al., 2004). The silk biopolymer is a cutting-edge substance for modern medicine, but even after many centuries, it is still regarded as a suture material. In order to meet present and future biomedical demands, we will be able to create new "old materials" as we continue to unlock the mysteries of silk (Holland et al., 2019).

Cantharidine

Cantharidine is a substance produced and released by the green blister beetle as a protection mechanism against other organisms. It is generated by the male beetle and supplied as a pre-copulatory gift to the female,

who uses it to protect her eggs (Sharma and Singh, 2018). Cantharidine and its derivatives have shown the anti-cancer activity against diseases like leukemia, colorectal carcinoma, hepatoma, bladder carcinoma, and breast cancer (Ratcliffe, 2006). Warts have been treated with cantharidine in clinical trials, where the blistering skin patches return to their prior condition without leaving a scar during the scarring condition in the patient (Maglio et al., 2003). Due to cantharidine's effectiveness in treating cancer, it has been widely used in clinical practise (Zhang et al., 2017). Cantharidine was approved by FDA in 2004 as a therapy for skin condition (warts) and to prevent cancer of kidney, urogenital tract, and ovary (Ratcliffe et al., 2011).

Antimicrobial peptides (AMPs)

Antimicrobial peptides (AMPs) are short proteins which are effective against parasites, fungi, viruses, and bacteria. They support an insect's inherent defences. Most of the known AMPs were first discovered in insects. AMPs from insects represent a potential source of substitute antibiotics in the light of the emergence of antibiotic resistance (Bulet et al., 2004). In 1980, the first AMP was isolated from giant silk moth pupae, and its bactericidal properties were made known for the first time (Hultmark et al., 1980). Currently, more than 1500 AMPs have been identified in a variety of organisms, including plants, fungi, bacteria and mammals. Insects are the main source of AMPs. In traditional Chinese medicine over 1700 different treatments have been created from about 300 bug species (Zylawska and Wyszynska, 2011). Identification challenges, drug toxicity, development costs, and large-scale production are all barriers to the evolution of insect compounds into potential modern medicines (Srivastava et al., 2009). Insect AMPs are divided into three classes based on their structure and amino acid sequence. Among them, Cecropins are present which are linear peptides with a helix but lack cysteine residues. Second most common AMPs in insects are Defensins which have 6–8 conserved cysteine residues, a stabilising array of 3 or 4 disulfide bridges, and three domains consisting of a flexible amino-terminal loop. In the end, Peptide Cecropins, drososocin, attacins, diptericins, defensins, ponericins, drosomycin, and metchnikowin are the most studied insect AMPs (Divyashree et al., 2020).

Maggot therapy

Maggot therapy, also known as larval therapy, is a treatment option for chronic, infected, necrotic and sloughy wounds (Rafter, 2012). The maggots employed in the therepy are the special clinical-grade, aseptically bred maggots of greenbottle fly larvae (*Lucilia sericata*). If it is decided that a wound qualifies for maggot treatment, the therapy can go forward under medical supervision (Hancock et al., 2012). The larvae used in maggot therapy clear the incision of infection as well as dead, sloughy, or necrotic tissue over the course of several days. Maggots are regarded to be beneficial to heal the wounds in three ways as they cause debridement (the removal of dead tissue), disinfection (minimising bacterial infection and biofilm load) of wound which ultimately assists in acceleration of wound healing (Malekian et al., 2019). In 2004, the use of medicinal maggots was approved by Food and Drug Administration (FDA) as a medical device to treat the wounds.

Insects as models

Fruit fly (*Drosophila melanogaster)*

There are various genetic and physiological similarities between insects and mammals. So, the insects are well suited as models for genetics, development, behavior and neurobiology. For this purpose they can be easily housed and bred without need of sophisticated equipment (Jans et al., 2021). The common fruit fly, *Drosophila melanogaster,* is a genetic model organism that has undergone extensive research and is very tractable for examining the molecular causes of human illnesses (Yamamura et al., 2021). *Drosophila* is employed as a model organism because of its many distinguishing characteristics. The fly genome has been entirely sequenced and annotated out of its total four chromosomes, three chromosomes carry the majority of the genome that have been encoded for more than 14,000 genes. It is believed that functional orthologs of almost 75% of the disease-related genes in humans exist in the fruitfly (Lloyd and Taylor, 2010). The overall degree of similarity between a fruitfly and a mammal's nucleotide or protein sequences is typically around 40 % in conserved functional domains, it can be as high as 80 to 90% higher. The fly has a relatively short life cycle. In just 10 to 12 days at 25°C, a single healthy mating pair can produce hundreds of genetically identical offspring (Adams et al., 2000). According to its developmental stage, the fly can be separated into a number of model organisms, including an embryo, a larva, a pupa, and an adult, each of which has a unique set of advantages (Reiter et al., 2001). Basic developmental research typically uses the embryo to examine pattern formation, cell fate determination, organogenesis, neural development and

axon path discovery. The larva, especially the third instar wandering larva, is commonly used to study physiological and developmental processes as well as certain basic behaviours like foraging behaviour (Brakel and Blumenthal, 1977).

Red flour beetle (*Tribolium castaneum)*

The red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) is a universal insect pest of stored grains. There are various applications and strengths of *Tribolium* beetles which are utilised for research in ecology and evolution (Pointer et al., 2021). *Tribolium* is an important model because its mode of short germ band development is more representative than other insect models (Adamski et al. 2019). It has been utilizing food sources varying in nutritional quality (Ming and Cheng, 2012). Therefore, these beetles are suitable for studying the effect of complex food items on longevity for the identification of food–gene interactions affecting stress tolerance (Adamski et al. 2019). The use of *Tribolium* beetles have been done for food safety and functionality studies which ultimately reduce risk to consumer health and economic losses (Piras et al., 2016).

Honey bee (*Apis mellifera*)

Previously, the use of honey bees as models of social behavior, aging and developmental plasticity has been practiced (Shpigler et al., 2017). The honey bee (*Apis mellifera*) gut microbiota is used as model for understanding microbiota functions. There are several advantages of honey bee gut microbiota as it sets up experimental system for understanding the effect gut communities on their hosts. It helps to understand the processes and routes governing particular gut communities impacting host biology (Zheng et al., 2018). Honey bees provide microbiota-free hosts which aids in understanding the mechanism of microbiota influencing host phenotypes along with disease states (Ridaura et al., 2013).

Silk worm (*Bombyx mori)*

Silkworm (*B. mori)* are also used as an experimental animal because of low rearing cost and a few ethical problems (Matsumoto and Sekimizu, 2019). There are various advantages of using silkworm models such as easy rearing, large size (Ishii et al., 2016). The use of silkworms as experimental models have been practiced for pathogens infecting humans (Miyazaki et al., 2012). Matsumoto and Sekimizu (2019) have been reviewed that silkworm based model have been established for various yeast type fungi such as *Candida albicans, C. tropicalis, C. glabrata* and *Cryptococcus neoformans.* Similarly, silkworm infection models have been established for filamentous fungi such as *Aspergillus fumigatus, Arthroderma vanbreuseghemii, Arthroderma benhamiae, Microsporum canis, Trichophyton rubrum* and *Rhizopus oryzae.*

Therapeutic use of insects

A study has been performed by Kasozi and co-workers (2020). In China, it was observed that local beekeepers were immune against Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Melittin and phospholipase A2 (PLA2), associated with bee venom have anti-inflammatory properties which shows activity against a large number of enveloped and non-enveloped viruses such as H1N1 and HIV. For the management of respiratory and neurological diseases, bee venom (BV) therapy has been used commonly as it involves group III secretory phospholipase. BV activation of the cellular and humoral immune systems should be explored for the application of complementary medicine for the management of SARS-CoV-2 infections. So, the workers concluded that BV previously showing efficacy against HIV and H1NI provides opportunity as a complementary therapy for protection against SARS-CoV-2 and there is a need to explore the activation of the cellular and humoral immune systems by bee venom in order to develop complementary medicine against SARS-CoV-2 infections.

Several previous studies explained that cantharidin is an inhibitor of protein phosphatase 2A (PP2A) which is responsible for controlling cell cycle, apoptosis (cell death), and cell-fate determination. Li et al. (2010) performed an experiment to investigate the curative effect of cantharidin in pancreatic cancer. The workers observed that cantharidin inhibits G2/M cell-cycle which leads to apoptosis and increased the expressions of pro-apoptotic tumour necrosis factors. The workers also observed that the inhibitory role of cantharidin is independent of oxidative stress in pancreatic cancer cells. This process ultimately inhibits the growth of pancreatic cancer cells without causing any effect on normal pancreatic duct cell.

In North East India, *Brachytrupes orientalis* (Gryllidae) is an edible insect species in many tribes. Dutta and co-workers (2019), conducted an experiment to explore the potentiality of *B. orientalis* against diabetic mellitus as an antioxidant and glucose metabolizing potential in different extracts solvents. On

supplementation with hydro-alcoholic (AEBO) solvent, glucose utilisation against high glucose exposure increased significantly in high glucose treated muscle cells. This leads to reduction of intercellular ROS production, lipid peroxidation and hike the protein expression of Nrf2 and GST. Thus, this study suggests the beneficial effect of AEBO on glucose metabolism in diabetes.

Commercial products

A number of insect based products has been developed for the treatment of various ailments and are commercially available in the market. Currently, various companies are developing honey products for the treatment of various ailments such as dry eye symptoms, wounds, minor abrasions, lacerations, minor cuts, minor scalds and burns, diabetic foot ulcers, and burns. Several FDA approved formulations of manuka honey are commercially available in the form of gels, wound dressings, eye drops etc (Hossain et al., 2021). Honey bee pollen or propolis products are available as nutritious supplements, anti-depressant, appetite modulator, as well as tonics to increase endurance in sporting activity, royal jelly as an antibacterial and antifungal agent and is commercially available in the form of capsules (Wang et al., 2016). The products obtained from blister beetles are available in the market under the trade name of cantharone plus developed by Canada based Dormer laboratories for the treatment of warts and canthradine hair oil is manufactured by many pharmaceutical companies in India. Silk scaffolds are available in various forms such as fibrous, porous, and hydrogel for tissue engineering (Babu, 2017). Laboratory reared sterile medical grade maggots has been used for wound healing. In China black ants products are available as powder, capsule, liquid extract or phoenix formula as they are a rich source of zinc, trace minerals and proteins (Tang et al., 2015).

Challenges in adoptoping insects as medicines

Despite all the benefits of using insects as drugs, there are several constraints encountered in the promotion of insects for therapeutic use in both developing and developed countries. This may be due to negative cultural attributes towards insects, particularly in the West (Imathiu, 2020). In Western countries, the barriers for developing modern medicines are more difficult to overcome as compared to countries like China, Brazil with a long tradition in the use of plant and animal extracts as medicines (Ratcliffe et al., 2011). In this area even today, scientific pioneers working is lacking due to political and public pressure in mounting for such drugs (Bordet, 2020). Even with the advancement of modern technology, the majority of insect species, such as crustaceans, go unaddressed for the presence of various natural products in comparison to other insect species, such as ants, beetles, butterflies, moths, and spiders. This uneven knowledge of the structures and functions of insect secondary metabolites is a result of the uneven distribution of insect species. With the exception of a few sex pheromones, the biological roles of even the chemically examined natural compounds have not been thoroughly investigated (Rather et al., 2020). On the basis of several clinical trials, it has been undertaken that use of insect based products such as manuka honey expands the healing times as compared to another synthetic drugs (Tonks et al., 2007; Cherniak, 2010) along with some side effects such as increased granulation tissue in wounds treated with honey or mild to moderate superficial burns (Moran, 1999). Insects can be the carrier of for certain hazards such as chemical substances (venom, antinutrients), parasites, viruses, bacteria and their toxins or fungi etc. In order to reduce potential risks of their consumption specific standards are lacking. Consumer acceptance is another obstacle mainly in the developed countries where insects are regarded in disgust by majority of the population (Lange and Nakamura, 2021).

CONCLUSIONS

Insects appear to be a virtually limitless resource for pharmaceutical study because of the protective compounds they've acquired during millennia of co-evolution with plants and other fauna. Exploitation of animal resources for therapeutic reasons has both ecological and cultural implications. It is concerning that there are a few of insect based items available in the market, despite the fact that many insect natural compounds show significant potential as sources of novel medications. In order to sustain the advantages of insects and products generated from them as an alternative in preventative medicine, safety measures such as quality control for commercial operations, reductions in pesticides, and barcoding for taxonomy are required. Insect variety must be preserved in order to supply future biological diversity and ingredients for new pharmaceutical research in the coming years. These topics should be explored in scientific symposia on conservation biology, public health, resource sustainability, biological prospecting, and patent law. Insects zootherapeutic potential can contribute significantly to the biodiversity debate, as well as lead

to the economic and cultural valorization of species that are typically dismissed as worthless. We must pay close attention to the long-term usage of insects.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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ANTIMICROBIAL PEPTIDES: PRINCIPAL DEFENSE CONSTITUENTS OF SILK WORMS - A REVIEW

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ABSTRACT

Insect has a highly developed innate immune system consisting of cellular and humoral responses. Antimicrobial peptides (AMPs) are the important components of the insect's humoral defense system and were first purified from the giant silk moth, *Hyalophora cecropia* L (Boman et al., 1980). For a variety of infections, they serve as the initial line of defense. AMPS are low molecular weight peptides and generally cationic in nature. Off late, the resistance of the pathogenic microbes towards established antibiotics have become a serious threat to global health. Over the years, an array of AMPs from natural sources has served as potent candidate against various infective agents viz. bacteria, fungi and viruses. These insect peptides not only exhibit antibacterial action by rupturing the microbial membrane but also prevent the development of drug resistance by microbes. Studies have shown AMPS to have synergistic effects with traditional antibiotics, providing an opportunity for combined therapy. Defensin, cecropins, drosocin, attacins, diptericins, ponericins, metchnikowins and melittin are a few classes of AMPs from insects that are currently isolated. However, the possibility of discovering new AMPs cannot be fully ruled out. Currently, 33 peptides are undergoing clinical trials, and 43% of the 77 AMPs are still in the preclinical stage (Makwana et al., 2023). Daptomycin and ovitavancin are two antibacterial lipopeptides that Food and Drug Administration has recently approved that are used for skin infection caused by bacteria. The present paper is an attempt to provide a comprehensive insight on antimicrobial peptides isolated from silk worm with reference to their structures, functions and possible mechanism of action.

Key words: Antimicrobial peptides, silk worm, *Philosamia ricini*, insects' immunity, cecropin, lebocin, moricin, attacin, defensin, gloverin, enbocin, isolation, characterization, microbes, multiple drug resistance

Silk worm belonging to the class Lepidoptera are one of the significant economic insects employed in molecular research (Goldsmith et al., 2005). Eri silk worm were known to be the earliest domesticated economic insects (Meng et al., 2017). They produce large offspring within a brief period of time, with larval stages lasting about 25-30 days (Fig. 1) and make silk which is a high-quality textile (Li et al., 2019). Sericulture is one of the major sources of income for many farmers in rural areas of India, China and Brazil (Wan et al., 2012). Besides the silk production, many immunological studies have been carried out on the bioactive properties of AMPs that seem to exhibit different mechanism with lesser side effects. Silk worms are being used as research model to study and understand the insect immunity and how better it can be implemented on human immune responses (Ravindar et al., 2015; Buhroo et al., 2018). One of the biggest risks to global public health is antimicrobial resistance. Antibiotic abuse in human and animal healthcare, particularly in the majority of developing countries, has caused threat in recent decades (Huan et al., 2020; Manniello et al., 2021; Ramzah et al., 2023). Scientists have predicted that antibiotic-resistance disease have the ability to kill millions of people per year and could cost the downfall of economy more than $100 trillion (Makwana et al., 2023). Development of new classes of antimicrobial drugs has slowed down over the past 35 years, which makes this undertaking much more challenging. As a result, there is an urgency of production and identifications of new antibiotic classes with therapeutics potential for treating variety of infectious disease in both humans and animals (Makwana et al., 2023).

Recently, screening of natural compounds with a wide range of chemical variations as significant and dependable sources of novel therapies is being pursued with interest (Uddin et al., 2021). One such class of naturally occurring substances is the AMPs, which are crucial elements of the innate immune system and are produced by variety of organisms in response to pathogenic stimuli (Dini et al., 2022).



Fig. 1. Life cycle of *Philosamia ricini* L

Several established AMPs such as moricin, cecropin, gloverins, attacin etc. were reported to be found in insects, mammals, reptiles and plants. The silkworm has a number of benefits over other insects in the field of life science, including low breeding costs, huge offspring sizes, rapid generation time, and distinct genetic backgrounds (Meng et al., 2017). The discovery of gramicidin from a *Bacillus* bacterium strain in 1939 by Rene Dubos opens a way to isolate and characterized numerous AMPs (Huan et al., 2020; Moretta et al., 2021). AMPs from insect can inhibit Multiple drug resistance (MDR) bacteria such as *Escherichia coli, Klebsiella poneumoniae, Strptococcus sanguinus and Staphylococcus aureus*. A small number of AMPs from insects also displayed antiviral efficacy against the herpes simplex virus type 1 and the human influenzaa A and B viruses. Numerous fungal strains, including *Aspergillus* species, *Bptrytis* sp, *Cryptococcus* sp. and *Fusarium* sp. have also been documented to be vulnerable to AMPS from insects (Manniello et al., 2021). In antimicrobial peptide database nearly 3500 AMPs were registered from various taxa (Mahlapuu et al., 2020). Seven AMPs have been approved for use by the U.S Food and Drug Administration and are marketed as of a research report from 2020 (Chen and Lu, 2020). In addition to medicine related fields, AMPs are also reported to use in industries, including food processing, animal husbandry, aquaculture and agriculture (Erdem and Kesmen, 2022).

A. Insects and immune system

Studies reveal the rapid advancement in the research of human toxicology and pathology using the silkworm as a model animal. Several studies observed that silkworms are extremely susceptible to pesticides, antibiotics, pathogenic fungus and human pathogenic bacteria. There are established silkworm models for bacteria, fungal, viral, and natural immunological activation (Kaito and Sckimizu, 2007; Ishii et al., 2015a). So, the current Research area of interest has been using the silkworm as a model organism to study human malignancy, degenerative diseases and metabolic diseases has been gaining momentum (Meng et al., 2017).

An insect lacks adaptive immunity, yet they have an efficient and sophisticated innate immune system to distinguish and obliterate the powerful microbes (Cheng et al., 2008; Ravi et al., 2011). Structural barriers such as epidermis, midgut peritrophic membrane and tracheal respiratory organs along with haemocoel acts as the first line of defense against microbes (Tanaka et al., 2008). Their innate immune system comprises of cellular and humoral defense mechanism (Buhroo et al., 2018). In cellular immunity of insects, direct interactions between circulating hemocytes and microbes through phagocytosis, nodulation and encapsulation takes place (Rizki and Rikzi, 1984; Dularay and Lackie,

1985; Ratcliffe et al., 1985; Gupta, 1986; Boman and Hultmark, 1987; Gupta, 1991). Hemocytes are classified into five types based on their morphology: prohemocytes (Pr), granulocytes (Gr), plasmatocytes (Pl), spehrulocytes (Sp) and oenocytes (Oe) (Strand, 2008; Tan et al., 2013; Ribeiro and Brehelin, 2006; Lavine and Strand, 2002). In insects, prohemocytes are largely found in hematopoietic organs (HPOs) and have the ability to differentiate into other types of hemocytes (Minakhina and Steward, 2010). Plasmatocytes and granulocytes recognize the foreign substances and activates its immune responses by the process of phagocytosis (Lavine and Strand, 2002; Dubovskiy et al., 2016). Oenocytes are rich in prophenoloxidases (PPO) and are mostly engagaed in melanization mechanism, while spherulocytes functions are still to be discovered (Makwana et al., 2023). In humoral immunity, certain peptides known as antimicrobial peptides are produced in insect hemolymph upon microbial infection (Bulet et al., 1999). The fat body and occasionally, other immunocompetent tissues such as epithelium, midgut epithelium, and hemocytes are the key sites where the humoral immune responses is initiated (Pradeep et al., 2013; Jayaram et al., 2014; Lekha et al., 2014; Lekha et al., 2015; Makwana et al., 2021). This immunity involves the activation of signaling pathways like Toll, Immunodeficiency (Imd), and Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways in immune-responsive tissue and cells of host to create AMPs and other effector molecules (Makwana et al., 2023).

B. Antimicrobial peptides

Antimicrobial peptides are present in almost all organisms from vertebrates to invertebrates (Mastore et al., 2021). It was first purified from the immunized pupae of a giant silk moth, *Hyalophora cecropia* by Boman's group (Hultmark et al., 1980; Steiner et al., 1981). AMPs are low molecular weight proteins, which are heat stable, typically cationic and often less than 100 amino acid residues that are encoded by genes and synthesized by ribosomes having broad range of activities against the invading microbes (Ganz, 2003; Wang and Lai, 2010; Buhroo et al., 2018). Their deter-genic properties have the ability to disrupt the cell membranes of the intruding microbes and enzymatically attacks bacteria by hydrolyzing their cell wall which is made up of peptidoglycan (Dunn, 1986; Russell and Dunn, 1996). AMPs not only inhibit the bacterial growth, instead some of them cease the growth by interfering with protein synthesis, nucleic acid synthesis, cell division, or protease activity (Vanzolini et al., 2022). Insect fat bodies are responsible for production of AMPs (Brey et al., 1998; Hoffmann et al., 1999; Ganz, 2003; Schmid-Hempel, 2005).

According to different studies, AMPs are biologically active peptides vital for humoral immune response of insects against micro-organisms (Imura et al., 2007; Lemaitre and Hoffmann, 2007; Song et al., 2015). Not only in insects, many of the AMPs been discovered from other species having antibacterial, antifungal and antiviral activities (Wu et al., 2018). Some peptides of AMPs even have anticancer properties (Jin and Winberg, 2018). AMPs are active towards gram-positive and gram-negative bacteria (Tanaka et al., 2008). Additionally, they are also noted to be effective against fungi (Noorwala et al., 1994) and protozoa (Aley et al., 1994) showing minimal inhibitory concentrations (Hancock and Lehrer, 1998). Owing to its broad range of activity, lesser toxicity and low resistance against target cells, they are considered as probable candidates for the forthcoming drugs (Buhroo et al., 2018).

Numerous antibacterial proteins have so far been identified from several insect species (Cociancich et al., 1994a), and they can be divided into five main categories (Hultmark et al., 1980; Hultmark, 1993). They are Cecropins, insect defensins, attacin resembling proteins which are rich in glycine, proline-rich peptides and lysozymes. In immunized hemolymph of *B. mori*, peptides viz. cecropins, lysozyme and proline-rich proteins have been reported (Chadwick and Aston, 1991; Hara and Yamakawa, 1995). Seven different kinds of AMPs (cecropins, drosocin, attacins, *dipteracins*, defensin, drosomycin and metchnikowins) have been isolated, identified and characterized from *Drosophila* (Bulet et al., 1999; Irving et al., 2001; Hetru et al., 2003). Defensins, cecropins, proline-rich peptides and attacins are commonly found in insects, while gloverins and moricins are reported only in Lepidopteran insects (Yi et al., 2014). Fruit flies and mosquitoes belonging to the order Diptera produces AMPs such as defesin, attacins, cecropins, lebocins, gloverins, moricin, drosomycin and drosocin while only few have been identified and isolated from Lepidopteran species such as *B. mori, Antheraea mylitta, A. assamensis, A. pernyi* and *Manduca sexta* (Hara and Yamakawa. 1995; Dutta et al., 2016; Souhail et al., 2016; Nayak et al., 2017; Zhao et al., 2018).

C. Lepidopteran antimicrobial peptides

1. Cecropins

Cecropins are the cationic polypeptides first isolated from the hemolymph of a giant silk moth, *H. cecropia* (Steiner et al., 1981; Hultmark et al., 1982). In insects three kinds of cecropins are present, i.e., A, B and D which lacks strongly basic cysteine with a flexible glycine-proline link connecting the strong basic N-terminal to the neutral C-terminal with a length of 35-37 residue (Moore et al., 1996). Cecropins have a wide-spectrum of antimicrobial activities against different microbes and are widely studied (Bulet et al., 2004). According to previous studies, 30 cecropins were identified from *Papilionoidea* species (Wang et al., 2021). These peptides were also identified from silkworms *A. pyreni* and *B. mori* and many have been isolated from lepidopteran and dipteran insects (Buhroo et al., 2018). Cecropins are highly effective against bacteria irrespective of their types and to some extent it acts against some fungi (Buhroo et al., 2018). Bactericidin, lepidopteran and sarcotoxin are few other names of insect cecropin (Ouyang et al., 2015). Structurally related peptides of cecropin are shown in Table 1. Three distinct forms of cecropin were detected in insects: cecropin A (37 amino acids), cecropin B (35 amino acids) and cecropin D (37 amino acids). Genomic sequence of the silkworm revealed a total of 11 cecropin genes, which have been divided into 5 sub-types: A constituting Bmcec A1 (2 genes), B (6 genes), C (1 gene), D (1 gene) and E (1 gene) (Cheng et al., 2006; Yang et al., 2011; Nesa et al., 2020).

a) Cecropin A: Cecropin A shows the stable helical structure (Fu et al., 2004). The regulatory motifs of Cec-A genes have been discovered since the 1990s after the genes were cloned (Yamano et al., 1998; Yamakawa and Tanaka. 1999). Antibacterial mechanism of cec-A was unknown, however there is strong evidence that it works by targeting the cell membrane (Silvestro and Axelsen, 2000). Reactive Oxygen Species (ROS) are created when cec-A dramatically lowers NADPH and glutathione levels, which further increases oxidative stress (Durell et al., 1992; Yun and Lee, 2016). In silkworm larvae, Cec-A exhibits potential efficacy against the fungus, *Beauveria bassiana* (Lu et al., 2016).

b) Cecropin B: It is a linear-cationic peptide consisting of 35 amino acid that occurs naturally with the highest level of antibacterial activity among the cecropin family (Srisailam et al., 2000). Cecropin B significantly decreased the plasma endotoxin and *E. coli* load mortality in a rat model of septic shock stages (Giacometti et al., 2001). The nematode worm *Brugia pahongi's* adult female motility were reduces by Cec B, while in *Aedes aegypti* adult female, cec-B significantly reduces the quantity of larval development (Chalk et al., 1995). Additionally, cecropin B exhibits antifungal properties against *Candida albicans* (Andra et al., 2001) and it has a wide range of activity against porcine bacterial infections (Hu et al., 2013). Cecropin B is a potent antibacterial compound that has been expressed using transgenic technology in the cocoon of silkworm (Liu et al., 2015). Similar to this, a transgenic breed of silkworm can over-expressed Cecropin B1 to produce silk with universal antibacterial action (Saviane et al., 2018). The confinement of *Xanthomonas oryzae pv. oryzye* infections were aided by transgenic expressions of silkworm cecropin B in rice (Li et al., 2019).

c) Cecropin C: The hemolymph of *H. cecropia* contains trace amounts of cecropin C that were formed by degradation of cec A. Antibacterial properties of cec C has not been widely documented (Hultmark et al., 1982).

d) Cecropin D: Cecropin D was isolated from the bacteria infected, *H. cecropia* that exhibits similarities

Table 1. Lists of amino acid sequences of cecropin A, B, B1, B3, C, D and P1. (Wu, Patocka and Kuca, 2018)

Antimicrobial peptides	Amino acid sequence	References
Cecropin A	GGLKKLGKKLEGVGKRVFKASEKALPVAVGIKALG-NH2	Steiner et al., 1981
Cecropin B	KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL-NH2	Steiner et al., 1981
Cecropin B1	KWKVFKKIEKMGRNIRNGIVKAGPKWKVFKKIEK-NH2	Srisailam et al., 2001
Cecropin B3	AIAVLGEAKALMGRNIRNGIVKAGPAIAVLGEAKAL-NH2	Srisailam et al., 2001
Cecropin C	GWLKKLGKRIERIGQHTRDATIQGLGIAQQAANVAATAR-NH2	Hultmark et al., 1982
Cecropin D	WNPFKELEKVGQRVRDAVISAGPAVATVAQATALAK-NH2	Hultmark et al., 1982
Cecropin P1	SWLSKTAKKLENSAKKRISEGIAIAIQGGPR-NH2	Pillai et al., 2005

to cecropin A and cecropin B (Hultmark et al., 1982). Cecropin D were discovered later than Cecropin A or Cecropin B following bacterial infection in the hemolymph of an insects (Gudmundsson et al., 1991). Successful expression of recombinant cecropin D in *Pichia pastoris* revealed antibacterial efficacy against both Gram-positive and Gram-negative bacteria (Guo et al., 2012). Due to active phosphorylation, cecropin D's C-terminal lysine residue have more antibacterial action (Park et al., 2013). Additionally, in vitro replication and infection of the porcine reproductive and respiratory syndrome virus (PRRSV) were inhibited by cecropin D (Liu et al., 2015).

e) Cecropin E: Cecropin E were reported from the lepidopteran insects with 35 amino acid residues. Both gram-positive and gram-negative bacteria are found to be susceptible to cec E (Hultmark et al., 1982).

Amidation at the C-terminus plays an important role in enhancing the antimicrobial property of cecropin and helps the cecropins- liposomes intereaction (Naikajima et al., 1987). The cecropin-derived substances Sarcotoxin-1 (Okada and Natori, 1985), Papiliocin (Kim et al., 2010), Stomoxyn (Boulanger et al., 2002), Hinnavin (Yoe et al., 2006), SB-37, and Shiva (Jaynes et al., 1988) are active against the parasites like *Plasmodium* and *Trypanosoma* (Arrowood et al., 1991; Barr et al., 1995; Boisbouvier et al., 1998). SB-37 and Shiva also has the ability to inhibit the development of malignant cells and the HIV-1 virus (Chen et al., 1997; Suttmann et al., 2008).

2. Defensin

Defensin, a family of peptides isolated from the hemolymph of immunized flesh fly, *Sacophoga peregrine* (Matsuyama and Natori, 1988). Another insect defensin were also isolated from the hemolymph of the dipteran insect, *Phromia terranovae* (Lambert et al., 1989). They are cationic in nature having 4 kDa of peptides (Buhroo et al., 2018) and comprises of six conserved cysteine (crucial characteristics of defensins) molecules as Sapecins (Matsuyama and Naturi, 1988) which forms the intra-molecular disulphide bonds (Xiao et al., 2004). Phormicins, royalcins, sapecins, and spodoptericis are some of the antibacterial defensins that have been isolated and characterized from the order Lepidoptera (Fujiwara et al., 1990; Yamada and Natori, 1993). They are effective against gram-positive bacteria like *Micrococcus luteus, Aerococcus viridians, Bacillus megaterium, B. subtilis, B. thuringiensis, and S. aureus* (Yi et al., 2014). Besides that, few of antifungal defensin were too reported: drosomycin from *Drosophila*, heliomycin from tobacco budworm *Heliothis virescens* (Lamberty et al., 1999), *Galleria mellonella,* a caterpillar of the larger wax moth, produced gallerimycin, and *Pseudacanthotermes spiniger*, an isopteran, produced termicin. (Lamberty et al., 2001b), and Alo13 from the order Coleoptera, harlequin beetle *Acrocinus longimanus* (Barbault et al., 2003). Insect defensins were isolated and reported from several insect orders such as dipteran, hymenopteran, coleopteran, trichopteran, hemipteran and odonata (Hoffmann and Hetru, 1992; Cociancich et al., 1994 b).

The hemocytes, silk gland, head, and ovary of the larvae as well as the fat body of the pupae were all shown to be strongly transcribed with the gene BmDefensinA, which was discovered from the genome of *B. mori*. This gene Bmdefensin regulates the immune challenges of the insect and play an important role in both immunity and metamorphosis of insects (Wen et al., 2009). A new defensin-like gene called Spodoptericin is modestly up-regulated by bacteria and fungus. It was observed that the sporoptericin gene lacked any sequence pattern that was clearly homologous to the cis regulatory element controlling antimicrobial peptide genes (Volkoff et al., 2003). When characterized BmDefA gene, it was reported that the gene contains 5' upstream regulatory region having three conserved regulatory sequence of cis elements i.e., NF-kβ binding site, IL-6 responsive element and GATA motif. This gene BmDefA when translate encode large peptides with N-terminal signal peptide (22 amino acid), a propeptide (34 amino acid) and a mature peptide (36 amino acid) with a molecular weight of 4 kDa. Mature BmDefA have the isoelectric point with a value of 4.2 leading to the discovery of novel anionic defensin (Buhroo et al., 2018; Nesa et al., 2020). BmDefensinB was also identified from the fat body of silkworm *B. mori* which acts as the homolog of insect defensin, with three NF-B binding sites and other cis elements (KAIKOBLAST). The findings imply that BmDefensinA and BmDefensinB contribute to *B. mori's* immunity (Kaneko et al., 2008). This defensin has only 27% of similarity in amino acid when compared to BmDefA and get activated by bacteria such as *E. coli* and *B. subtilis* and also by *Beauveria bassiana*. When used against *Pseudomonas aeruginosa* MDR strains, rabbit neutrophils defensins exhibit strong bactericidal actions (Zhao et al., 2005). Toll and Imd pathway control the gene expression of BmDefB and plays an important role in regulation of immune reaction in both bacteria and fungi in the organism *B. mori* (Buhroo et al., 2018). Amino acid sequence of defensin is shown in Table 2.

Table 2. Comparision of the gene BmDefensinB and BmDefensinA from *B. mori (*Kaneko et al., 2008)

BmDefensinB	--ALPCAK----KS-CDSWCRRLDYPCGECVTKWKCSCNNWMQ DK	38
BmDefensinA	---IWCEFEEATETAI CQEHCLPKGYSYGI CVSN-TSCCI-----	36

3. Moricin

Moricin are highly basic 42-residue peptide that was first isolated from the *B. mori* immunized with *E. coli* and thus peptide actively suppressed the growth of *S. aurens* (Hara and Ymakawa, 1995). Moricin has only ever been discovered in Lepidopteran insects, and cDNAs encoding it have been found in *M. sexta* (Zhu et al., 2003), *Spodoptera litura* (Oizumi et al., 2005), *G. mellonella* (Brown et al., 2008), *Helicoverpa armigera* (Wang et al., 2010b) and other insects. The cDNA sequences of *S. exigua*, *Heliothis virescens*, and *Hyblaea puera* are also available in the NCBI database (Yi et al., 2014). According to Christensen et al. 1988; Gabay, 1994, the basicity is assumed to be the cause of the attachment of positively charged peptides to the negatively charged bacterial surface through the electrostatic interaction. Moricin's isoelectric point was determined to be 12.0, greater than cecropin's (pI=8.2-9.6). Secondly, an amphipathic α-helix structure has been observed present in moricin that are often in charge of expressing antibacterial action (Hara and Yamakawa, 1995). *B. mori* genome have 12 moricin-coding genes that can be divided into three subtypes, i.e., Bmmor (1 gene), moricin-like A (3 genes) and moricin-like B (8 genes) and above all, Bmmor gene exhibits highest antibacterial activity against bacteria (Yang et al., 2011). In an in vitro experiment, moricin from *B. mori* (Bmmor) was found to have antibacterial properties against *E. coli, B. bombysepticus, B. subtilis, B. thuringiensis, B. thuringiensis galleriae, Serratia marcescens, S. aureus, P. aeruginosa,* and *Ralstonia solaanacearum* at dosages of 0.625-1.25 mL/L (Rd). Bmmor, a member of the *B. mori* moricin family, has the highest antibacterial toxicity (p 0.01) and is followed by the moricin-like A subtype. The B subtype of moricin, in contrast, has no effect on the tested microorganisms (Yang et al., 2011).

Genes encode with mature moricin, contain a positively charged C-terminus and an amphipathic alpha helical N-terminus without any post-translational modification paving a way for synthesizing moricin chemically (Islam et al., 2016). The α-helical motif of moricin plays an important role in increasing the membrane permeability to destroy pathogen (Yi et al., 2014). Charged amino acids can be seen in the N-terminal half of moricin at intervals of three or four amino acid residues, indicating a structure common to antibacterial proteins with the amphipathic α-helix (Cociancich et al., 1994a; Kreil, 1994). Both gram-positive and gram-negative bacteria are sensitive to moricins; and the moricins that was isolated from *G. mellonella* shows high activity against filamentous fungi and yeasts (Hara and Yamakawa, 1995a; Brown et al., 2008; Dai et al., 2008). Moricin have a significant role in *B. mori's* self-defense against bacterial infection since it was produced in response to bacterial infection and exhibits substantial antibacterial activity (Hara and Yamakawa, 1995).

4. Attacins

The peptide attacin with a molecular weight of 20-23 kDa are glycine-rich protein (Wu et al., 2018) and were first isolated from *H. cecropia* pupae (Hultmark et al., 1983; Buhroo et al., 2018). Attacin are found in both acidic and basic form with an isoelectric point ranging from 5.7 to 8.3 (Nesa et al., 2020). Six isolated attacin AMP were classified into 2 groups: a basic group constituted by attacin A to D and an acidic group comprising of attacin E and F (Buonocore at al., 2021). Acidic attacin has a high contain of aspartic acid, arginine and isoleucine, whereas basic attacin has a high contain of lysine, glutamic and tryptophan (Islam et al., 2016). Amino sequence of attacins A-F is shown in Table 3. Attacin shows antibacterial activity against *E. coli, Stenotrophomonas maltophilia* and *Acinetobacter calcoaceticus* (Nesa et al., 2020). Attacin blocks the synthesis of outer membrane proteins of gram-negative bacteria which disturbed the cell wall of bacteria causing a chain or long chain formation (Carlsson et al., 1998; Nesa et al., 2020). Attacin are third antibacterial protein found in the humoral immune system of *H. cecropia* after cecropin and lysozyme (Kockum et al., 1984).

Table 3. Amino-terminal sequence of attacin A-F. Their sequence difference can be observed from the highlighted residue (Wu et al., 2018)

Attacin A-C	AGALTINSDGTSGAVVKVPI- NH2
Attacin D-F	DAHGALTLNSDGTSGAVVKVPFAGNDLNI- NH2

5. Gloverin

Gloverin is a basic antibacterial protein with a molecular weight of ~14 kDa isolated from the pupal haemolymph of giant silk moth, *H. gloveri* (Axen et al., 1997; Buhroo et al., 2018; Nesa et al., 2020). This peptide contains 18.5% of glycine rich amino acid residue and lacks cysteine residue (Axen et al., 1997). This peptide is active against gram-negative and gram-positive bacteria and shows a bit similarity to attacin peptides (Kaneko et al., 2007). Pro-gloverin feature a conserved RXXR motif at their N-terminal region, and gloverins are produced as pre-pro proteins (Xu et al., 2012). The N-terminal pro-regions (22–26 residues) are likely eliminated by furin-like enzymes before the synthesized of mature gloverins. However, recombinant *Trichoplusia ni* pro-gloverin is equally as effective against *E. coli* as mature gloverin from *H. gloveri* (Lundstorm et al., 2002), indicating that some pro-gloverins may not require the removal of pro-regions in order to be active (Yi et al., 2014). Gloverin was said to expressed only in lepidopteran insects, namely *Helicoverpa armigera* (Mackintosh et al., 1998a), *T. ni* (Lundstrom et al., 2002), *A. mylitta* (Gandhe et al., 2006), *M. sexta* (Abdel-latief and Hilker, 2008; Xu et al., 2012), *Diatrea saccharialis* (Kawaoka et al., 2008; Mrinal and Nagaraju, 2008). *E. coli* infected silkworm produce this peptide in the fat body of insects (Mrinal and Nagaraju, 2008) and detail study about their genome revealed that the silkworm produces 4 genes encoding Bmglv 1,2,3 and 4 (Yang et al., 2011). Among them, *B. mori* gloverin 1 is the ancestral gene and gloverin 2-4 are produce by the duplication of gloverin 1 gene (Mrinal and Nagaraju, 2008). In insect *B. mori*, Gloverin 1 were found to be expressed in the gonads of larval pupae of silkworm but not in the adults, while gloverin 2-4 are expressed only on adults' gonads of silkworm (Mrinal and Nagaraju, 2008). In *M. sexta,* this peptide is highly expressed in the testis of naive larvae (Xu et al., 2012). Detailed studies about gloverin peptide leads to the conclusion that these peptides not only have the antimicrobial activity but also plays an important role in the development and reproduction of insects (Yi et al., 2014).

6. Lebocin

Lebocin were initially discovered as proline-rich, O-glycosylated 32-residue peptides in the hemolymph of *E. coli* immunized silkworm *B. mori* (Hara and Yamakawa, 1995b). According to a complementary DNA clone for *B. mori* lebocin, the active 32-residue peptide is situated adjacent to the C-terminus of the precursor protein, which contains 179 residues (Choudhury et al., 1995). The amino acid residue (15-Thr) is a crucial component of antibacterial action (Islam et al., 2016). These peptide fundamental structure and antibacterial properties mostly match with those of abaecin (41% of same amino acid sequence) of honey bees that have 34-residue of the proline-rich peptide and devoid of O-glycosylated (Casteels et al., 1990).

Lebocin 1 and lebocin 2 are two distinct analogues of lebocin that share identical amino acid residue but differ in the length of their sugar chains on the threonine residues. According to reports, these antibacterial proteins with glycosylated threonine include various sugars, such as galactose, N-acetyl galactosaminein lebocin 1 and N-acetyl galactoseamine in lebocin 2. Amino acid sequence of lebocine 3 showed one amino acid replacement at 16-Leu and 15-Thr. Lebocin amino acid sequence shows similarity with other members of lebocin (Islam et al., 2016). Amino acid sequence of lebocin is shown in Table 4. In vitro research discovered that lebocin promotes lipid bilayer leaking underneath low ionic circumstances that could indicate active rupture of bacterial membrane (Hara and Yamakawa, 1995). Due to its low sensitivity and the requirement for a low ionic conductivity, however, its specific mechanism is still being studied. Lebocin 3 has an additive cecropin D, as their conjugation significantly boosts the cecropin D's antimicrobial effects on *B. mori* (Ponnuvel and Yamakawa, 2002).

7. Enbocin

Enbocin is a novel antimicrobial peptide having 59 amino acid residues identified from *B. mori* (Kaneko et al., 2007). In 1998, a silkworm cDNA fragment was cloned to screen the first gene expression named as enbocin. After performing an experiment, it was reported that the amino acid sequence of enbocin belongs to cecropin family (Kim et al., 1998). Although, their antibacterial properties differ from that of cecropin

Table 4. Amino acid Sequences of Lebocin (Nesa et al., 2020)

Lebocin 1	DLRFLYPRGKLPVPTPPPENPKPIYIDMGNRY
Lebocin 2	DLRFLYPRGKLPVPTPPPENPKPIYIDMGNRY
Lebpcin 3	DLRFLYPRGKLPVPTPPPENPKPIYIDMGNRY
Lebocin 4	DLRFLYPRGKLPVPTPPPENPKPITIDMGNRY

(Kim et al., 1998). This enbocin exhibits antibacterial activity against gram-positive and gram-negative bacteria. In silkworm, at least 2 enbocin genes are present (Kaneko et al., 2007). According to the analysis of induction mechanism, the expression of silkworm enbocins is induced by the bacteria *E. coli* and thereafter occurs mostly in the fat body (Li et al., 2019). Amino acid sequences were shown in Table 5.

CONCLUSIONS

Insect AMPs exhibit antibacterial action against a variety of microorganisms. Despite their small size, AMPs are becoming increasingly popular due to their incredible qualities and capabilities. Because they can generate more potent and stable peptides, smaller peptides of insect AMPs are regarded to be a superior candidate for medical applications. Silkworms have recently gained popularity in the field of life sciences for applications such as environmental safety monitoring and pharmaceutical pathogen screening. As the number of diseases resistant to more than one antibiotic increases, the efficacy of several medications falls. This motivates scientists to seek out new sources of bioactive chemicals that naturally destroy germs. Silkworm AMPs has focused mainly on antibacterial properties, severely limiting their use in other domains. A thorough examination of the role and application of silkworms in the manufacture of AMP is necessary. The silkworm model has been applied successfully in numerous domains of life science study, significantly improving scientific research in this area. Many hurdles remain, and the application of the silkworm model in many disciplines is still in its infancy due to a lack of evidence from clinical trials and adequate animal investigations.

Finally, boosting the use of silk worm models in scientific research will bring new insights into long-held problem-solving assumptions, helping both science and society greatly. This model organism offered a bright future to the researchers. The activity of silk worm AMPs determines their relevance for applications. Silk worm AMPs have numerous applications, including biomaterials, pharmaceuticals, food bio preservation, and disease prevention in plants and animals. According to medical professionals, silk worm AMPs are promising antibiotic candidates that can be transformed into powerful anticancer and antiparasitic medications. The discovery and classification of AMPs, an important effector of the innate immune system, are significant in demonstrating how AMP variations can be developed to combat the increased prevalence of multi-drug resistant infections as a viable alternative to traditional antibiotics. This study highlights silk worm as a potential source of antimicrobial peptides and opens up new channels for intervention and the creation of naturally occurring bioactive chemicals to combat antibiotic resistance.

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Table 5. Amino acid sequence of Enbocin, Enbocin 2 and Enbocin 3 (Kaneko et al., 2007)

Enbocin	MNFTRIIFFLFVVVFATASGKPWNFKEIERAVARTRDAVISAGPA RTVAAATSVASG
Enbocin 2	MNFTRIIFFLFVVVFATASAKPWNFFKEIERAVARTRDAVISAGPAVATVGAAAAVASG
Enbocin 3	MNFTRIIFFLFVVVFATASAKPWNFFKEIERAVARTRDAVISAGPAVATVAAASAVASG

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THE EVOLUTION OF EUSOCIALITY: INSIGHTS FROM COMPARING TWO INDIAN PAPER WASP SPECIES

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ABSTRACT

Eusocial species live in colonies with a reproductive division of labour into fertile reproductive castes and sterile non-reproductive castes, an overlap of generations and cooperative brood care. A further distinction can be usefully made between primitively eusocial species which do not have morphological caste differentiation and highly eusocial species which do. *Ropalidia marginata* is a tropical primitively eusocial wasp that has been extensively studied, especially compared to other tropical social wasps. *R. marginata* has several distinct traits, such as a docile queen, well-developed age polyethism, and decentralized work regulation, which makes it different from other primitively eusocial wasps and reminiscent of highly eusocial species. Since tropical wasps, especially those belonging to the genus *Ropalidia* have been poorly studied, we cannot be sure whether *R. marginata* is unique or its traits are more common among tropical *Ropalidia* species. To begin to overcome this problem, we have extended our research to the congeneric and sympatric *Ropalidia cyathiformis*. Here, we compare and contrast what we now know about these two species, especially concerning their reproductive and non-reproductive division of labour. We find that *R. cyathiformis*, unlike *R. marginata*, has a behaviourally dominant queen, weak and rigid age polyethism, likely uses behavioural dominance to regulate worker reproduction and individual workers self-regulate their own non-reproductive activities. We, therefore, conclude that *R. marginata* is indeed unique and argue that *R. marginata* is intermediate between primitively and highly eusocial wasps.

Key words: *Ropalidia marginata*, *Ropalidia cyathiformis*, primitively eusocial, highly eusocial, dominance behaviour, social wasps

Many animals, especially insects, live in social groups and depend on each other for survival and reproduction. The extent of social interaction and integration vary widely. To focus on the most social species, the term eusocial, originally coined by Suzanne Batra (Batra, 1966), is used for species that display reproductive caste differentiation into fertile reproductives (queens and kings) and sterile non-reproductives (workers), an overlap of generations and cooperative brood care (Michener, 1969; Wilson, 1971). In addition to the division of reproductive labour between queens and workers, there is often a further division of non-reproductive labour among subsets of workers, although the extent of the latter may vary greatly.

Based on the reproductive division of labour, eusocial insects are classified as primitively eusocial, with no morphological differentiation between reproductive and non-reproductive and highly eusocial insects, where the reproductives, and non-reproductive are morphologically distinct. In the highly eusocial species reproductive caste differentiation occurs in the pre-imaginal (larval) stage as a process of developmental regulation so that adults are quite fixed in their roles at eclosion. In the primitively eusocial species, by contrast, reproductive caste differentiation largely occurs in the post-imaginal (adult) stage by a process of social regulation, leading to considerable flexibility in the roles that an individual can adopt.

All termites and all ants (except perhaps the queenless ants in the sub-family ponerinae), honey bees and some wasps are considered highly eusocial while many bees and wasps are primitively eusocial. Among the wasps, examples of highly eusocial species include most epiponine and vespine wasps (Wilson, 1971; Ross and Matthews, 1991) while examples of primitively eusocial species include all polistine wasps and bumble bees (Fletcher and Ross, 1985; Wheeler, 1986; Gadagkar et al., 1988; O'Donnell, 1998).

Flexibility in the social roles of adults in the primitively eusocial species results in the potential for conflict among individuals for the position of a reproductive in the colony. Studies on the mechanism

of queen succession and determinants of becoming a queen in primitively eusocial wasps have revealed age and dominance behaviour as the two most important factors in predicting whether an individual becomes a queen or not (Pardi, 1948; Yoshikawa, 1963; West-Eberhard, 1969, 1978; Jeanne, 1972; Litte, 1979; Dew and Michener, 1981; Strassmann and Meyer, 1983; Miyano, S., 1986; Hughes and Strassmann, 1988; Giannotti and Machado, 1997; Suzuki, 2003). In addition, the queens in such primitively eusocial societies appear to use dominance behaviour to maintain their reproductive monopoly in the colony. A clear exception to this rule is the primitively eusocial wasp *Ropalidia marginata,* where the queen is docile (Gadagkar and Joshi, 1983; Chandrashekara and Gadagkar, 1992; Gadagkar, 2001; Sumana and Gadagkar, 2003) and uses pheromones to regulate reproduction amongst the workers of her colony. The usage of chemicals to regulate reproduction is a trait that is more commonly observed in highly eusocial species (Winston and Slessor, 1992; Keller and Nonacs, 1993; Katzav-Gozansky et al., 2002; D'Ettorre et al., 2004; Monnin, 2006)1993; Katzav-Gozansky et al., 2002; D Ettorre et al., 2004; Monnin, 2006). However, recent studies have shown that perhaps the usage of pheromones for maintaining reproductive control might not be restricted to just highly eusocial species and used by some primitively eusocial species as well (Dani and Turillazzi, 2018; da Silva et al., 2020; Tsuchida et al., 2020; Oi et al., 2021).

Non-reproductive division of labour could be based on morphology, with individuals of different sizes performing different tasks, as in the case of termites and several species of ants (Oster and Wilson, 1978; Noirot, 1989; Hölldobler and Wilson, 1990) or based on age as in the case of honey bees, stingless bees and several species of ants (Oster and Wilson, 1978; Seeley, 1982; Sommeijer, 1984). In the age-based division of labour or age polyethism, individuals perform different tasks as they age, with younger individuals performing tasks within the colony such as nursing and maintenance of the nest and older individuals doing tasks outside the colony such as foraging. In the case of primitively eusocial insects, since there is no morphological differentiation among individuals, an age-based division of labour is expected. Studies on primitively eusocial insects reveal a rudimentary and not very well-developed age polyethism, although it has been explored relatively less (Jeanne, 1991). Again, an exception to the rule is the primitively eusocial wasp *Ropalidia marginata*, which has a strongly developed and flexible age polyethism similar to what is observed in highly eusocial insect species such as honey bees and stingless bees (Naug and Gadagkar, 1998a, 1998b).

Ropalidia marginata therefore, emerges as a species of interest, considering its similarities to highly eusocial insects despite being primitively eusocial (Fig. 1). However, one cannot yet draw rigorous conclusions regarding the levels of sociality of *R. marginata* for two main reasons - 1) the study methodology between *R. marginata* and other primitively eusocial wasps differ greatly, making the comparisons difficult and 2) the majority of studies in other primitively eusocial wasps are mainly on temperate wasps, whereas *R. marginata* is a tropical species. Temperate and tropical wasps have important differences in their nesting cycles, with the former having an annual colony cycle and the latter a perennial cycle (Reeve, 1991). These different life cycles lead to several specific adaptations. In the temperate species for example, reproductives are produced only at certain times of the year and are different in size with more fat deposits from other workers, reducing flexibility in their social roles (Haggard and Gamboa, 1980; Strassmann, 1981; Reeve, 1991). Mated female wasps overwinter in hibernacula and start new colonies the following spring. In tropical wasps on the other hand, nests can be initiated throughout the year, and individuals do not differ in morphology. Hence, any worker is potentially capable of becoming a queen, resulting in higher flexibility in social roles.

The best and only way to work around these difficulties and understand whether *R. marginata* is perhaps at a level of sociality between primitive and advanced societies, would be a comparable study, with



Fig. 1. Picture of *Ropalidia marginata*

similar methodology, focussed on another tropical primitively eusocial species. We have therefore begun to focus our attention on the tropical primitively eusocial wasp *Ropalidia cyathiformis*. *R. cyathiformis* (Fig. 2) is a congeneric species to *R. marginata* and shares the same habitat range (Gadagkar and Joshi, 1982; Kardile and Gadagkar, 2005). *R. cyathiformis* possess an aggressive and dominant queen (Gadagkar and Joshi, 1982, 1984; Deshpande et al., 2006), already indicating that it might be similar to its more studied temperate counterparts. These features make *R. cyathiformis* a suitable model system to compare and contrast with *R. marginata* and the other temperate paper wasps. In this review, we compare and contrast the results and inferences of three separate studies on *R. cyathiformis* on the reproductive (Unnikrishnan and Gadagkar, 2017) and non-reproductive division of labour (Unnikrishnan and Gadagkar, 2020) as well as on the regulation of division of labour (Unnikrishnan and Gadagkar, 2021) with similar studies in *R. marginata*. We also discuss how the two species compare with other primitively eusocial wasps.

2. Behaviour of the queens

2.1. *R. cyathiformis*

Although there is no morphological differentiation between queens and workers in primitively eusocial species, the behaviour of the queens is nevertheless of particular interest because they are usually the sole egg layers of the colony. Gadagkar and Joshi (1984) constructed time-activity budgets for all individually identified wasps in colonies of *R. cyathiformis*. Subjecting these data to principal components analysis and cluster analysis, they demonstrated the presence of three rather distinct clusters of wasps. Based on their behavioural profiles, they called these clusters 'behavioural castes' and labelled the 3 clusters as sitters, fighters and foragers (Fig. 3). Sitters spend most of their time sitting on the nest, apparently doing nothing. Fighters most frequently indulge in dominance-subordinate interactions with colony members.



Fig. 2. Picture of *Ropalidia cyathiformis*
(Photo courtesy: Dr. Thresiamma Varghese)

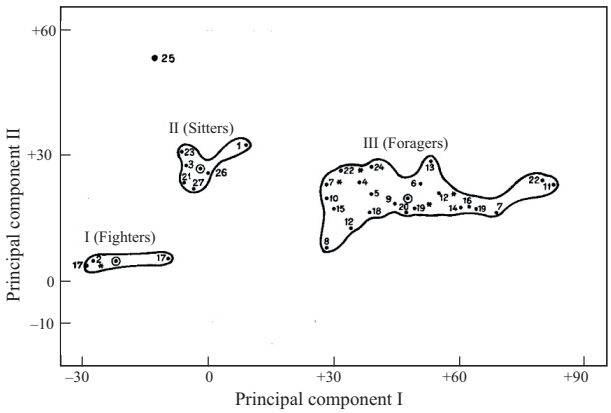


Fig. 3. Behavioural clusters of *R. cyathiformis*. Time-activity budget of 32 wasps is shown as points in the coordinate space of the first two principal components. The points fall into the 3 clusters by the criterion of the nearest centroid. Circled dot = centroid (Reproduced with permission from Gadagkar and Joshi, 1984)

In contrast, foragers spend more time away from the nest than the other behavioural castes, bringing back food and building material. Since the queens were not treated any differently during this analysis, the researchers could post-facto identify the behavioural caste of the queens. The *R. cyathiformis* queens were found to belong to the fighter caste. This was not surprising, since queens of primitively eusocial wasps are known to be aggressive individuals and use dominance behaviour to regulate the activities of the workers (Pardi, 1948; West-Eberhard, 1969; Jeanne, 1972; Litte, 1979; Strassmann, 1981; Strassmann and Meyer, 1983; Reeve and Gamboa, 1983; Miyano, S., 1986; Yamane, 1986; Hughes and Strassmann, 1988; Keeping, 1990; Giannotti and Machado, 1997; Tindo et al., 1997; O'Donnell, 1998; Tindo and Dejean, 1998; Giannotti, 1999; Noda et al., 2001; Suzuki, 2003; Prezoto et al., 2004; Oliveira et al., 2014). Consistent with this, Kardile and Gadagkar (2002) showed that *R. cyathiformis* queens are consistently at the top of the dominance hierarchies of their colonies. All subsequent studies have repeatedly re-confirmed that *R. cyathiformis* queens are active and interactive, aggressive fighters, usually at the top of the dominance hierarchies of their colonies (Deshpande et al., 2006; Gadagkar, 2001; Unnikrishnan and Gadagkar, 2017, 2021).

2.2. *R. marginata*

Gadagkar and Joshi (1983) constructed time-activity budgets for all individually identified wasps

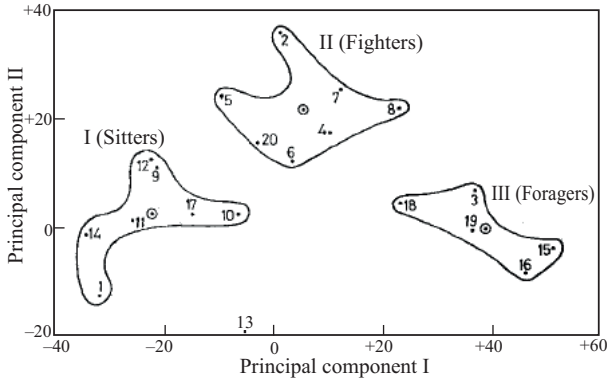


Fig. 4. Behavioural clusters of *R. marginata*. Time-activity budget of 20 wasps is shown as points in the coordinate space of the first two principal components. The points fall into the 3 clusters (castes) by the criterion of the nearest centroid. Circled dot = centroid (Reproduced with permission from Gadagkar and Joshi 1983)

in two colonies of *R. marginata*. Subjecting these data to principal components analysis and cluster analysis, they demonstrated the presence of three rather distinct clusters of wasps, sitters, fighters and foragers, based on their behavioural profiles (Fig. 4). It might have been expected that the queens also belonged to the fighter caste in this species, as in the case of *R. cyathiformis*. Instead, it turned out that the queens of both colonies of *R. marginata* belonged to the sitter caste. Chandrashekara and Gadagkar (1991a) re-confirmed this surprising result using 12 additional colonies. Chandrashekara and Gadagkar (1991) also constructed dominance hierarchies for each colony and showed that queens in *R. marginata* are seldom at the top of the dominance hierarchy. *R. marginata* queens have sometimes been found to be at the top of the dominance hierarchies of their colonies in small colonies, but this has been shown to be due to chance alone where the probability of any individual being on the top of the hierarchy is high (Chakraborty et al., 2018). All subsequent studies have repeatedly re-confirmed that *R. marginata* queens are meek and docile sitters, seldom at the top of the dominance hierarchies of their colonies, rarely interacting with the workers (Chandrashekara and Gadagkar, 1992; Gadagkar, 2001; Sumana and Gadagkar, 2003).

3. Reproductive division of labour

3.1. *R. cyathiformis*

Most colonies of *R. cyathiformis* contain a single egg layer, although workers have been seen to lay 1 or 2 eggs on rare occasions, despite the queen's presence. However, it is not clear whether it should be

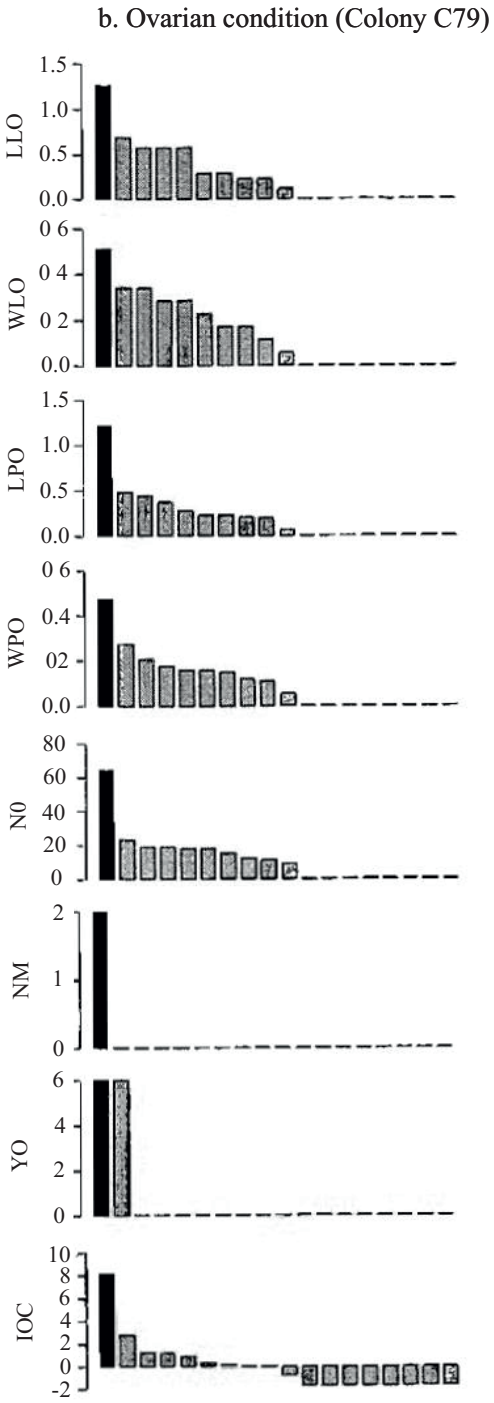


Fig. 5. All the seven measurements of ovarian development are given. Grey bars represent workers while black bars represent queens. LLO, length of the largest oocyte; WLO, width of the largest oocyte; LPO, average length of proximal oocytes; WPO, average width of proximal oocytes;.NO, total number of oocytes; NM, number of mature oocytes; YO, number of oocytes with yolk; IOC, composite index of ovarian development (Reproduced with the kind permission of the Bombay Natural History Society from the "Journal of the Bombay Natural History Society – Vol 102: 266–273)

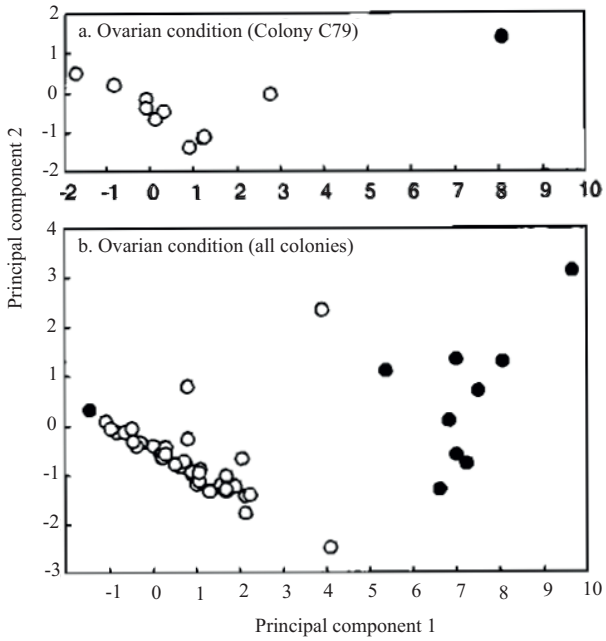


Fig. 6. Intra- and inter-colony variation in ovarian condition (a) representative colony and (b) all colonies. Closed circles = queens, open circles = workers (Reproduced with the kind permission of the Bombay Natural History Society from the "Journal of the Bombay Natural History Society – Vol 102: 266–273)

treated as rare evidence of egg-laying workers or the presence of multiple queens in this species (Gadagkar and Joshi, 1982). In the absence of morphological caste differentiation between queens and workers, the difference between the labels 'egg-laying workers' and 'multiple queens' may only be semantic. The near reproductive monopoly of the queen is confirmed by data on ovarian development showing that queens have the best-developed ovaries, by any measure of ovarian development, and that workers seldom reach the queen's level of ovarian development even when compared across colonies (Kardile and Gadagkar, 2005) (Figs. 5, 6).

It is interesting to note, however, that workers often have some low level of ovarian development, suggesting that they are not permanently sterile but that their ovarian development is suppressed in the presence of the queen. If this is true, workers should be able to develop their ovaries and lay eggs if the suppressing effect of the queen is removed. This prediction has been borne out by the observation that, upon experimental removal of the queen, one of the workers develops her ovaries and takes over the role of the queen as the sole, or near-sole egg-layer of the colony (Deshpande et al., 2006; Unnikrishnan and Gadagkar, 2017). There are several remarkable features of such queen succession. Upon removal of the queen, one and only one worker becomes hyper-aggressive within a few hours, displaying high levels of dominance behaviour towards almost all the remaining workers, and she is almost never the recipient of any dominance behaviour from her nestmates (Deshpande et al., 2006; Unnikrishnan and Gadagkar, 2017). This hyper-aggressive 'potential queen' develops her ovaries and begins to lay eggs within 1–9 days (mean± SD = 5.2± 3.5) (Unnikrishnan and Gadagkar, 2017). The potential queen (PQ) reduces her aggression at about the time she lays her first egg, and she subsequently remains about as aggressive as is typical for an *R. cyathiformis* queen.

The fact that the PQ is never challenged by the rest of the workers in the colony indicates that perhaps she has been pre-designated in some way to be the next queen of the colony. Indeed, studies show that there is a reproductive queue of potential queens who replace their predecessor queens in a remarkably conflict-free process of queen succession (Unnikrishnan and Gadagkar, 2017) (Figs. 7, 8). The reproductive queue, however, is not very long and ends with the 2nd (1 colony) or 3rd potential queen (10 colonies); no subsequent hyper-aggressive PQs were immediately identifiable, and observations have therefore been usually terminated at this point (Unnikrishnan and Gadagkar, 2017). The dominance behaviour shown by an individual in the presence of the queen is a statistically significant predictor of whether an individual will become a queen and also of her position in the reproductive queue. No other parameter, such as age, ovarian status or body size, has any role in determining whether an individual would become a queen or not, nor in determining the position of an individual in the queue. However, even dominance behaviour is not a perfect predictor because the next most dominant individual does not always become the next queen (Unnikrishnan and Gadagkar, 2017). Instead, potential queens are drawn from among the most dominant workers in the colony, but the order in which this subset of individuals succeed the dying or removed queens of the colony, cannot be predicted.

Nevertheless, dominance behaviour is important in queen succession as it is the only significant predictor of the identity of the potential queen. And this is not surprising as in all primitively eusocial species studied so far, dominance behaviour is an important predictor of whether or not an individual becomes a queen. On the other hand, it is surprising that age is not a significant predictor of the identity of the potential queen. In most primitively eusocial wasps studied, age is important in queen succession. In the

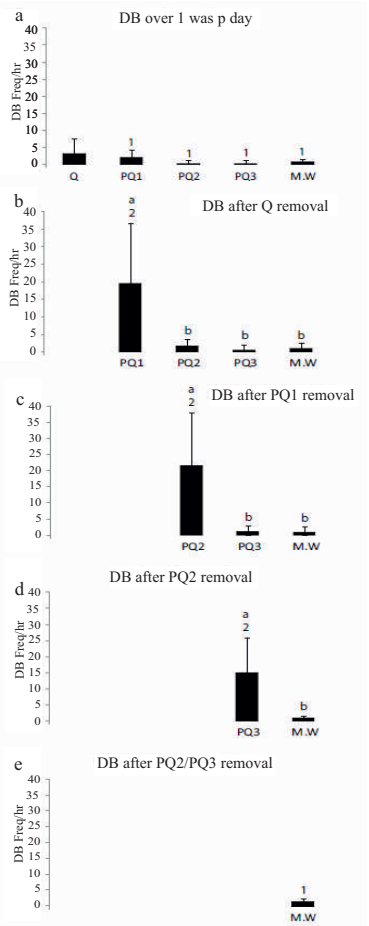


Fig. 7. Reproductive queue in *R. cyathiformis*. The mean and standard deviations of the frequencies per hour of dominance behaviour of the queen, 3 PQs and M.W (max worker, which is the individual who showed the highest frequency of dominance behaviour other than the Q and all three PQs), in normal queen-right colonies (a) and in the absence of the queen and the preceding PQs (b–d) (n = 12 for a–c and 10 for d and 8 for b). Bars carrying different alphabets are significantly different from each other within each panel (Wilcoxon matched-pairs, signed-ranks test; to achieve $P<0.05$, α was set to 0.008, 0.01 and 0.05 on account of Bonferroni correction for b, c and d, respectively). Bars carrying different numbers show the significant difference in the frequency per hour of dominance behaviour shown by the focal PQ in the queen-right session with itself after the removal of the queen or previous PQs (Wilcoxon matched-pairs, signed-ranks test; to achieve $P<0.05$, α was set to 0.01 on account of Bonferroni correction). All the PQs showed significantly higher dominance behaviour after the removal of the queen and the previous PQs compared to itself in the presence of the queen. Also, after the removal of PQ3 (and in two nests after PQ2) there was no identifiable PQ4. The dominance shown by the M.W was not significantly different from the M.W in the queen-right session. Also there was no significant difference in aggression shown between the M.W and the next most dominant individual in the colony (data not shown, Wilcoxon matched-pairs, signed-ranks test; $P<0.05$). DB dominance behaviour, Q queen, PQ1–PQ3 potential queens 1–3, M.W max worker (Reproduced with permission from Unnikrishnan and Gadagkar 2017)

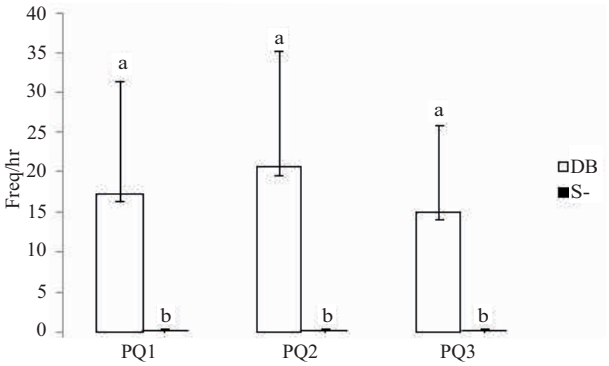


Fig. 8. Mean and standard deviations of dominance behaviour shown and received by PQs, after the removal of queen/previous PQs. Bars carrying different alphabets are significantly different from each other for each PQ (Wilcoxon matched-pairs, signed-rank test; $P<0.05$; n = 19, 15 and 10 colonies each for PQ1, PQ2 and PQ3). DB dominance behaviour, S- dominance received, PQ1–PQ3 potentials queens 1–3. (Reproduced with permission from Unnikrishnan and Gadagkar 2017)

so-called 'temperate pattern' of queen succession, the oldest wasps in the colony become future queens, as in the case of *Polistes dominulus* (Hymenoptera: Vespidae) (Pardi, 1948; Taylor et al., 2020), *P. jadwigae* (Hymenoptera: Vespidae) (Yoshikawa, 1963), *P. fuscatus* (Hymenoptera: Vespidae) (West-Eberhard, 1969), *Mischocyttarus. flavitarsis* (Hymenoptera: Vespidae) (Litte, 1979), *P. metricus* (Hymenoptera: Vespidae) (Dew and Michener, 1981), *P. exclamans* (Hymenoptera: Vespidae) (Strassmann and Meyer, 1983), *P. chinensis antennalis* (Hymenoptera: Vespidae) (Miyano, 1986), *P. annularis* (Hymenoptera: Vespidae) (Hughes et al., 1987), *P. instabilis* (Hymenoptera: Vespidae) (Hughes and Strassmann, 1988), *P. lanio* (Hymenoptera: Vespidae) (Giannotti and Machado, 1997), *Belonogaster petiolata* (Hymenoptera: Vespidae) (Keeping, 1997), *Liostenogaster flavolineata* (Hymenoptera: Vespidae) (Bridge and Field, 2007) and *Mischocyttarus cassununga* (Hymenoptera: Vespidae) (Murakami and Shima, 2009). In the so-called 'tropical pattern' of queen succession, relatively younger wasps supersede the queen, as in the case of *Polistes canadensis* (Hymenoptera: Vespidae) (West-Eberhard, 1969), *Mischocyttarus drewseni* (Hymenoptera: Vespidae) (Jeanne, 1972), *Metapolybia aztecoides* (Hymenoptera: Vespidae) (West-Eberhard, 1978) and *Parapolybia indica* (Hymenoptera: Vespidae) (Suzuki, 2003).

3.2. *R. marginata*

In thousands of hours of observation by dozens of observers, workers in *R. marginata* have not been seen to lay eggs in the presence of a healthy queen. Hence, to

the best of our knowledge, the queen is always the sole egg layer of the colony (Chandrashekara and Gadagkar, 1991b; Gadagkar, 2001; Gadagkar et al., 1982). This is consistent with all available data on the ovarian development of queens and workers. Only queens have fully developed ovaries by any measure of ovarian development, while workers have significantly under-developed ovaries. Even when queens and workers from different colonies are considered simultaneously, the ovarian development of queens is significantly better than that of workers (Figs. 9, 10) (Chandrashekara and Gadagkar, 1991b; Gadagkar, 2001).

As in *R. cyathiformis*, upon experimental removal of the queen, one of the workers develops her ovaries and takes over the role of the queen as the sole egg-layer of the colony, in *R. marginata* also (Chandrashekara and Gadagkar, 1992; Kardile and Gadagkar, 2002; Premnath et al., 1996; Sumana and Gadagkar, 2003). The process of such queen succession has been studied in more detail in *R. marginata*. There are several remarkable features of queen succession in this species. Immediately upon removal of the queen, one and only worker becomes hyper-aggressive, displaying high levels of dominance behaviour towards almost all the remaining workers, but she herself is almost never the recipient of any dominance behaviour from her nestmates (Chandrashekara and Gadagkar, 1992; Sumana and Gadagkar, 2003; Bang and Gadagkar, 2012). This hyper-aggressive 'potential queen' develops her ovaries and begins to lay eggs within 3-11 (5.62± 0.29) days (Lamba et al., 2007). The potential queen loses almost all her hyper-aggression and behaves like a docile sitter queen, characteristic of the species, at about the time she lays her first egg (Fig. 11) (Premnath et al., 1996; Sumana and Gadagkar, 2003). Although the identity of the potential queen becomes evident within minutes of removing the queen (sooner than in *R. cyathiformis,* where it takes several hours to identify the PQ), all attempts to predict her identity before removing the queen have so far failed. And yet, there is clear evidence that the potential queen is a 'cryptic heir designate' known to all the workers (Bhadra and Gadagkar, 2008). As in *R. cyathiformis*, there is a reproductive queue of potential queens who replace their predecessor queens in a remarkably conflict-free process of queen succession; indeed, the reproductive queue in *R. marginata* is even longer, extending at least up to 5 PQs, perhaps it is even longer (Figs. 12, 13) (Bang and Gadagkar, 2012). Potential queen/s cannot be identified in the presence of the queen because they are not unique by any criterion, not in their dominance

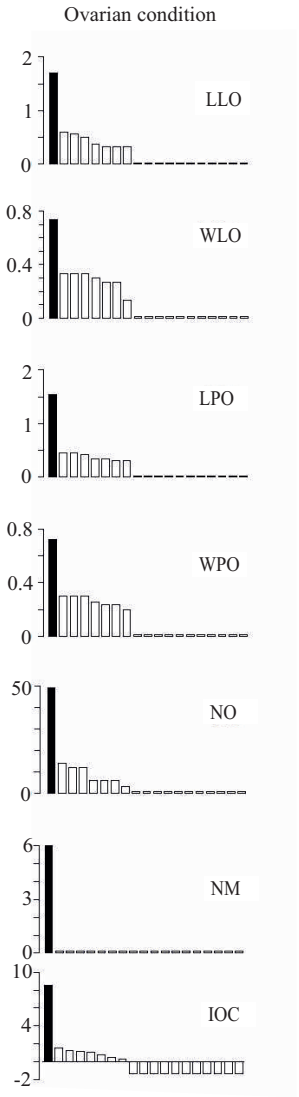


Fig. 9. Ovarian condition in *R. marginata*. The individual values used to assess ovarian condition are shown for all the workers (open bars) and the queen (black bars) in a typical colony. LLO, length of the largest oocytes; WLO, width of the largest oocyte; LPO, mean length of the proximal ooctyes; WPO, mean width of the proximal oocytes; NO, total number of oocytes; NM, number of mature eggs (proximal oocytes that, on microscopic examination, appeared to have a fully formed chorion, which gave them a characteristic pearly white appearance); and IOC, index of ovarian condition. For each panel, the values for different individuals are arranged in descending order. Each bar represents one individual. Note that the queen always has the highest value for all measures of ovarian condition; several individuals have intermediate values; and a large number of individuals (all those other than the queen for the number of mature eggs) have a value of zero. All measurements except total number of oocytes and number of mature eggs are in mm (Data from Chandrashekara and Gadagkar, 1991a.) (The social biology of ropalidia marginata: toward understanding the evolution of eusociality by Raghavendra Gadagkar, Cambridge, Mass.: Harvard University Press, Copyright © 2001 by the President and Fellows of Harvard College. Used by permission. All rights reserved.)

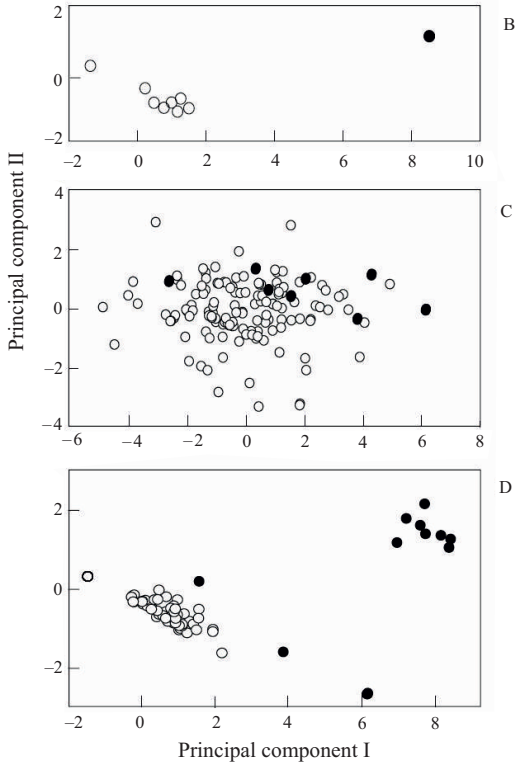


Fig. 10. B: Relative positions of queen and workers, with reference to their ovarian condition in a typical colony. Data from 5 are subjected to principal components analysis and the relative positions of the queens and workers are plotted in the space of the first two principal components. C and D: The relative positions of queens and workers from all the 11 colonies derived from a similar analysis. Note that queens and workers are well separated from each other. (Data from Chandrashekara and Gadagkar, 1991a) (The social biology of ropalidia marginata: toward understanding the evolution of eusociality by Raghavendra Gadagkar, Cambridge, Mass.: Harvard University Press, Copyright © 2001 by the President and Fellows of Harvard College. Used by permission. All rights reserved)

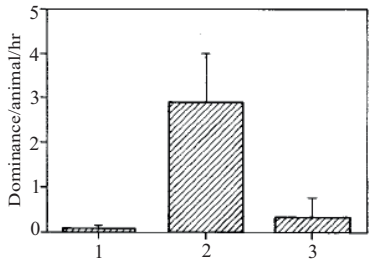


Fig. 11. Frequency of dominance behaviour of the founding queen, the individual next in the hierarchy on the day groups were formed and of the founding queen on the natal nest. Dominance acts per animal per h of a founding queen on the day groups were formed (2) is significantly greater than that of the wasp next in the hierarchy on this day (3) and than that exhibited by the founding queen earlier on the natal nest prior to nest-removal (1) (Wilcoxon matched-pairs signed-ranks test, $P < 0.001$ for 1 and 2; 2 and 3) (Reproduced with permission from Premnath et al. 1996)

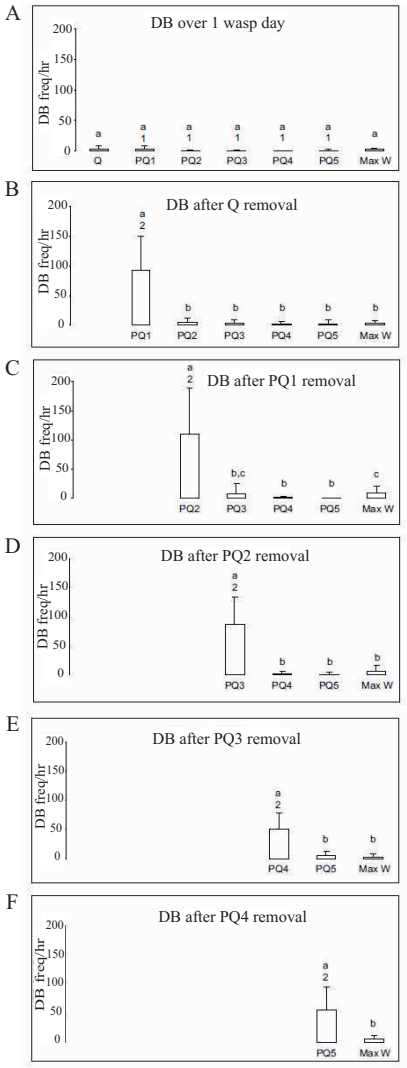


Fig. 12. Evidence for a reproductive queue in *R. marginata*. Means and SDs of frequencies per hour of behavioural dominance of the queen, five PQs, and the max worker (the individual that showed maximum behavioural dominance in the colony apart from the queen and the five PQs) in normal queenright colonies (A) and the PQs and the max workers in the absence of the queen and the preceding PQs (B–F) (n = 19 colonies). Bars that carry different letters are significantly different from each other within each panel (Wilcoxon matched-pairs, signed-ranks test; to achieve $P < 0.05$, α was set to 0.002, 0.003, 0.005, 0.008, 0.017, and .05 on account of Bonferroni correction, for A, B, C, D, E, and F, respectively). Bars that carry different numbers represent significant differences in freq/h of behavioural dominance of the focal PQ in normal queenright colony with her own value after removal of the queen or previous PQs (Wilcoxon matched-pairs, signed-ranks test; to achieve $P < 0.05$, α was set to 0.01 on account of Bonferroni correction). Note that each PQ showed significantly higher aggression after the queen and the previous PQs were removed than what she showed in the queenright colony and also compared with any other individual in the queenless colony. DB, behavioural dominance; Q, queen; PQ1 to PQ5, PQs 1–5; Max W, max worker (Reproduced with permission from Bang and Gadagkar 2012).

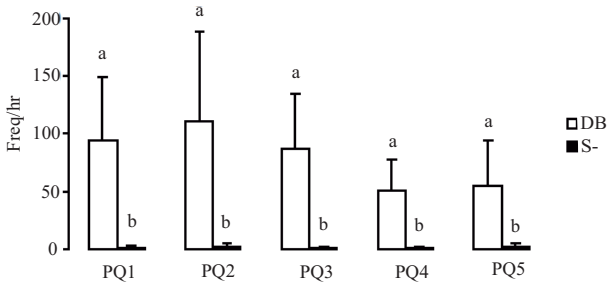


Fig. 13. Behavioural dominance shown and received (mean± SD) by different PQs in the absence of the queen and previous PQs (Wilcoxon matched-pairs, signed-ranks test; to achieve P < 0.05, α was set to 0.01 on account of Bonferroni correction; n = 19 colonies). Note that each PQ showed significantly higher behavioural dominance than what she received. On an average, dominance shown by each PQ was ~40–180-fold higher than the dominance she received. DB, behavioural dominance shown; S-, behavioural dominance received; PQ1 to PQ5, PQs 1–5 (Reproduced with permission from Bang and Gadagkar 2012)

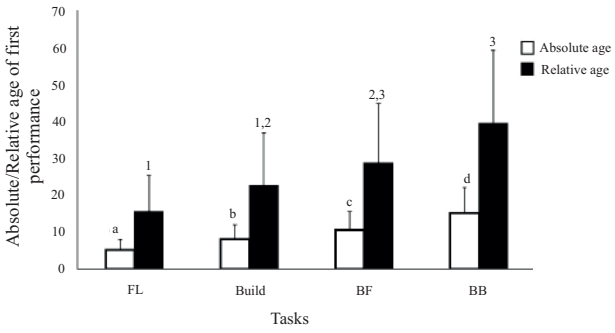


Fig. 14. Kruskal Wallis for both Absolute and Relative ages, absolute age – $\chi 2 = 35.4$, df = 3 P < 0.001; relative age – $\chi 2 = 15.5$, df = 3, P = 0.001. Mann Whitney U test showed significant difference between mean ages for successive tasks in absolute age (FL vs BU - U = 576, P = 0.001; BU vs BF – U = 332, P < 0.03; BF vs BB – U = 219, P = 0.03). Relative age showed no difference between mean ages for successive tasks (Mann Whitney U test, FL vs BU – U = 435, P = 0.14; BU vs BF – U = 298, P = 0.19; BF vs BB – U = 202, P = 0.12), but showed significant difference between, FL and BF and FL and BB and Build and BB (Mann Whitney U test, FL vs BF – U = 520, P = 0.003; BU vs BB – U = 228, P = 0.016). N = 100 (FL), 91 (Build), 44 (BF) and 26 (BB) (Reproduced from: Unnikrishnan, S., Gadagkar, R. (2020). The effect of age on non-reproductive division of labour in the tropicalprimitively eusocial wasp, Ropalidia cyathiformis. Int. J. Dev. Biol. 64, 267-273. https://doi.org/10.1387/ijdb.190213su - with the permission of UPV/EHU Press).

rank in the colony, not in their behaviour, nor their residual levels of ovarian development (Chandrashekara and Gadagkar, 1992; Deshpande et al., 2006). The lack of a role for dominance rank in determining the next queen makes *R. marginata* quite different from other primitively eusocial wasps, including *R. cyathiformis*. Here the age of workers is a statistically significant predictor of their position in the reproductive queue. But age is not a perfect predictor because it is not as if the next oldest individual always becomes the next queen; potential queens often jump the age-based queue (Bang and Gadagkar, 2012). Instead, potential queens are drawn from among the oldest workers in the colony and the order in which they succeed the dying or removed queens of the colony cannot be predicted. Hence, it appears that queen succession in *R. marginata* follows a temperate pattern despite being a tropical species. A reversal of the pattern of queen succession, though in the opposite direction, has also been observed in the temperate species, *Parapolybia indica*, where queen succession followed a tropical pattern despite being a temperate species (Suzuki, 2003).

4. Non-reproductive division of labour - age polyethism

4.1. *R. cyathiformis*

In highly eusocial insects such as honey bees, workers perform various non-reproductive tasks in an orderly manner, shifting from one task to another in a predictable sequence as they grow older. This is referred to as age polyethism (Lindauer and Watkin, 1953; Seeley, 1982; Winston, 1987). *R. cyathiformis* displays a rudimentary age polyethism compared to honey bees (Unnikrishnan and Gadagkar, 2020). Changes in the performance of the two important intranidal tasks, namely, building the nest and feeding the larvae, and two important extranidal tasks, namely, foraging for food and foraging for building materials, have been studied as a function of the age of the workers. These four tasks are performed by most individuals in the sequence - feed larva, build, bring food and finally bring building materials - as they age from about five days to about 60 days (Fig. 14). This trend of initiating intranidal tasks before extranidal tasks is common in other primitively eusocial wasps as well

(Yoshikawa, 1963; Dew and Michener, 1981; Jeanne, 1991; Tsuchida, 1991; Torres et al., 2012, 2013; Murakami et al., 2013; Cecílio et al., 2016). Task performance can be measured in two different ways. The frequency of task performance (FTP) is defined as the absolute frequency with which a task is performed. The probability of task performance (PTP) is defined as the probability of performing a task relative to other tasks. The age of an individual in the colony can be measured both as its absolute age in the number of days since eclosion and as its relative age with respect to the age distribution of the rest of the colony.

Both FTP as well as PTP vary with absolute

and relative age, although not always statistically significantly. Age explains the changes in FTP better than it explains the changes in PTP. The sequence of the task performance and their dependence on age mentioned above are valid both for absolute and relative age. However, absolute age is a better predictor of both FTP and PTP (Fig. 15). The best statistical fit is obtained when FTP is regressed against absolute age (Unnikrishnan and Gadagkar, 2020). In other words, the clearest evidence for age polyethism in *R. cyathiformis* comes from the observation that individuals modulate the absolute frequency of performing different tasks based on their absolute age. The frequency of performing intranidal tasks has a bell-shaped curve, with the maximum frequency obtained around middle age and then decreasing with age. The same pattern is observed in the case of extranidal tasks as well, indicating no clear-cut trade-off between performing intranidal tasks and extranidal tasks. Thus, individuals continue to perform both intranidal and extranidal tasks simultaneously (Unnikrishnan and Gadagkar, 2020). Taken together, we find that - (a) the division of labour is based on absolute age and not relative age, thereby reducing the flexibility of individuals to respond to changes in the colony demography and (b) the lack of trade-off between intra- and extranidal tasks suggests that individuals perform both simultaneously, indicating that the age polyethism in *R. cyathiformis* is not so well developed, as has also been observed in other primitively eusocial wasps (Yoshikawa, 1963; Dew and Michener, 1981; Jeanne, 1991; Tsuchida, 1991; Torres et al., 2009, 2012, 2013; Murakami et al., 2013). In the case of *Belonogaster petiolata* (Keeping, 1992) and *Mischocyttarus latior* (Cecílio et al., 2016), age polyethism appears to be completely absent.

4.2. *R. marginata*

As in the case of *R. cyathiformis*, there is clear evidence of age polyethism in *R. marginata*; indeed, age polyethism appears to be much better developed in this species (Naug and Gadagkar, 1998). In a slight departure from *R. cyathiformis*, most *R. marginata* workers perform tasks in the sequence - feed larva, build, bring building materials and finally bring food - as they age from about five days to about 60 days or more (Fig. 16). As in the case of *R. cyathiformis*, both FTP and PTP vary significantly with both the absolute and relative ages of the individuals. However, in contrast to *R. cyathiformis*, age explains changes in PTP better than it explains changes in FTP, and relative age is a

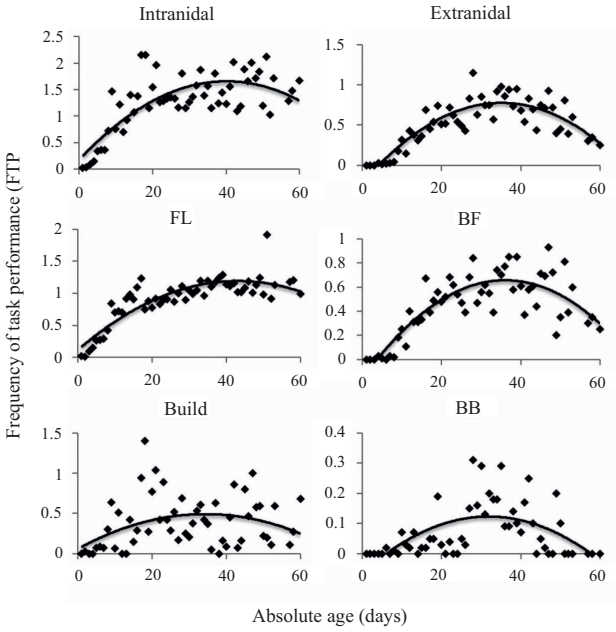


Fig. 15. Frequency per hour of task performance (FTP) as a function of absolute age fitted with second order polynomial regression lines. Data points represent the mean value (in frequency/ hr) for all individuals in that age class in 4 colonies (Reproduced from: Unnikrishnan, S., Gadagkar, R. (2020). The effect of age on non-reproductive division of labour in the tropical primitively eusocial wasp, Ropalidia cyathiformis. Int. J. Dev. Biol. 64, 267-273)

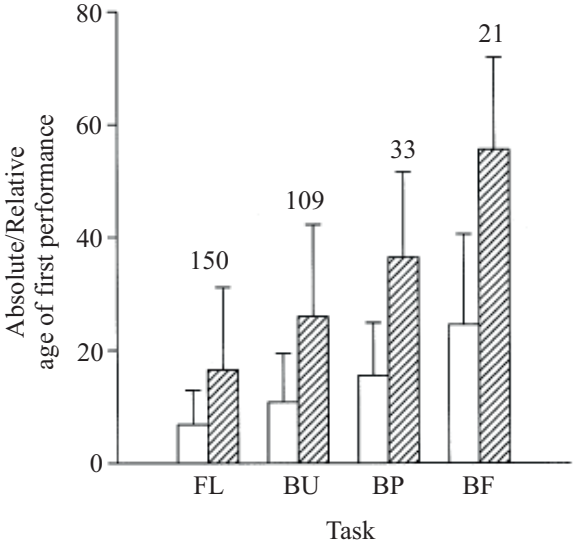


Fig. 16. Mean (+/ - SD) age of first performance for each task in terms of absolute age (open bars) and relative age (shaded bars). Sample size for each task is given above the respective bars. Multiple comparisons of mean ages of first performance using Tukey-Kramer method indicated significant differences across tasks ($p< 0:05$). The first performance of a task was significantly influenced by absolute age (one-way ANOVA, F 33:47, $P <$ 0:0001) and relative age (F 49:12, $P < 0:0001$). Mean ages for successive tasks were also significantly different ($p< 0:05$) by a Mann-Whitney U-test (Reproduced with permission from Naug and Gadagkar 1998a)

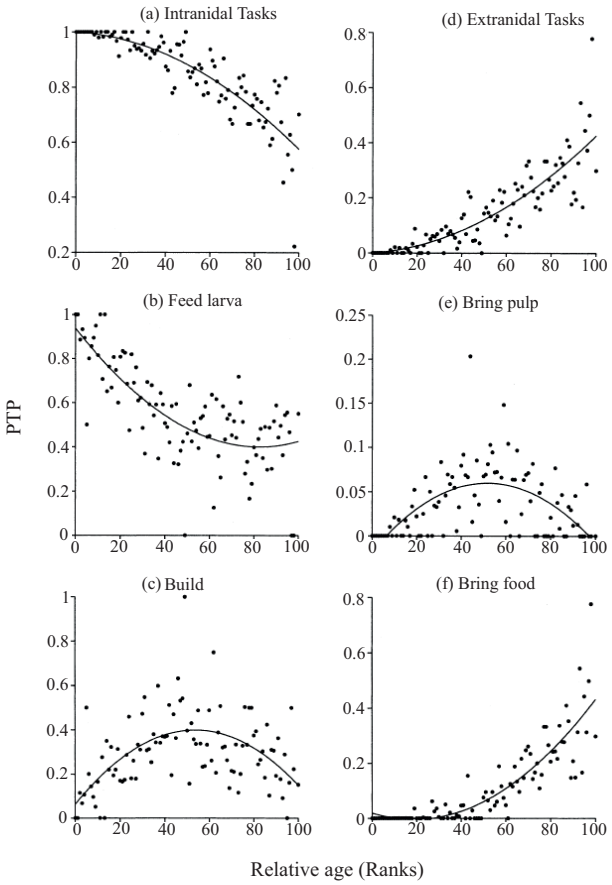


Fig. 17. Probability of task performance (PTP) as a function of absolute age (top) and relative age (bottom) fitted with second-order polynomial regression lines. Data points represent the mean value for all individuals in that age class in five colonies (Reproduced with permission from Naug and Gadagkar, 1998a)

better predictor of both FTP and PTP in this species (Fig. 17). The best statistical fit in *R. marginata* is obtained when PTP is regressed against relative age (Naug and Gadagkar, 1998). In other words, the clearest evidence for age polyethism in *R marginata* comes from the observation that individuals modulate the probability of performing different tasks, relative to other tasks, based on their age relative to the age of other members of the colony, thereby providing flexibility to respond to changes in the colony demography. In *R. marginata,* the probability of performing intranidal tasks decreases with age.

In contrast, the probability of performing extranidal tasks increases with age such that there is a clear trade-off between performing intranidal tasks versus extranidal tasks. In other words, individuals begin to perform extranidal tasks by gradually giving up intranidal tasks as they grow older (Naug and Gadagkar, 1998a). The well-developed age polyethism in *R. marginata* is in contrast to other primitively eusocial wasps where age polyethism, if it exists, is poorly developed.

5. Non-reproductive division of labour - foraging and feeding larva

5.1. *R. cyathiformis*

Queens and workers show a clear-cut division of labour in foraging; queens perform no foraging, and workers are entirely responsible for this task. There is, however, no such clear-cut division of labour in the case of feeding the larvae; this task is shared between the queens and workers, such that queens are similar to an average worker in the rates at which she feeds larvae (Kardile and Gadagkar, 2003). Since there is no clear-cut partitioning of intranidal and extranidal tasks between different workers, as seen above, both foraging and feeding larvae are performed by most of the workers. This is in contrast to the possibility that some workers forage and bring food, hand it over to intranidal workers who then feed the larvae, a situation seen in the congeneric *R. marginata* (see below) much more strikingly than in *R. cyathiformis*. In the case of the queen, however, she unloads food from incoming foragers, some of which she feeds to the larvae. It is interesting to note that the removal of the queen does not affect the rates at which workers bring food and feed the larvae (Kardile and Gadagkar, 2003; Unnikrishnan and Gadagkar, 2021) (Fig. 18).

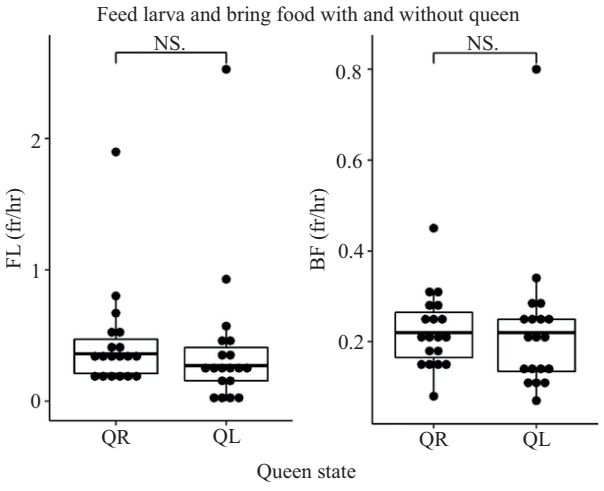


Fig. 18. Frequency per hour of feed larva (FL) (left panel) and bring food (BF) (right panel) behaviours in queen-right (QR) and queen-less (QL) sessions. There was no difference in the frequency of FL between QR and QL sessions (LMM − Estimate = 0.05, CI − 0.07 to 0.17, P value = 0.39) and also in frequency of BF between QR and QL sessions (LMM − Estimate = − 0.002, CI − 0.06 to 0.05, P value = 0.94). N = 19 colonies (Reproduced with permission from Unnikrishnan and Gadagkar, 2021)

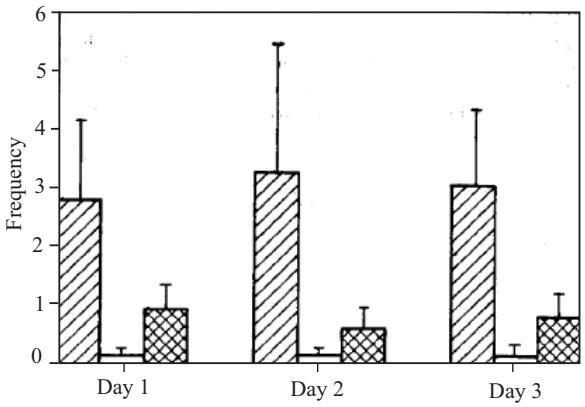


Fig. 19. Frequencies per hour of total food brought (hatched bars), food brought per animal per hour (open bars) and feed larvae per animal per hour (cross-hatched bars) are all not significantly different among days 1 (with queen), 2 (without queen), and 3 (after re-introduction of queen). Comparisons are by two tailed Wilcoxon matched-pairs signed-rank test (Reproduced with permission from Premnath et al. 1995)

5.2. *R. marginata*

As in *R. cyathiformis*, there is a clear-cut division of labour between queens and workers such that queens never forage and workers perform all the foraging tasks. Unlike in *R. cyathiformis,* however, there is also a division of labour in the task of feeding the larvae such that queens rarely feed larvae, and workers are responsible for performing most of this task. Among the workers, there is again a more clear-cut partitioning of intranidal and extranidal workers. Foragers or extranidal workers bring food to the colony, which is then unloaded by the intranidal workers (Premnath et al., 1995; Lamba et al., 2008). This food is then distributed amongst the other intranidal workers, who then feed the larvae. Similar to *R. cyathiformis*, removal of the queens results in no difference in the rate of workers foraging and feeding the larvae in *R. marginata* also (Premnath et al., 1995) (Fig. 19).

6. Regulation of reproductive division of labour

6.1. *R. cyathiformis*

Since the queen in *R. cyathiformis* is a behaviourally dominant individual, she might be expected to suppress reproduction by the workers by means of her dominance behaviour. A detailed examination of the colony's initiators and recipients of dominance behaviour should help verify this conjecture. It turns out that the queen preferentially directs her dominance behaviour to the potential queen, and the potential queen preferentially directs her dominance behaviour to the workers; neither the potential queen nor the workers show any dominance behaviour to the queen, nor do the workers show any dominance behaviour towards the PQ. It, therefore, appears that the queen may be regulating worker reproduction using her dominance behaviour, but she seems to do so via the PQ. Such a strategy of using the PQ to help maintain her reproductive monopoly in the colony may save her much time and energy, which can be channelled into her own reproduction (Unnikrishnan and Gadagkar, 2021). Although the use of dominance behaviour to regulate reproductive activities of other workers in the colony by the queen is common in other primitively eusocial wasps (Pardi, 1948; West-Eberhard, 1969; Jeanne, 1972; Litte, 1979; Strassmann and Meyer, 1983; Hughes et al., 1987; Hughes and Strassmann, 1988; Keeping, 1992, 1997; Giannotti and Machado, 1997), the strategy of utilizing the PQ for this task is something that has not been reported before. Whether this is because it is not present or because it has not been studied yet is unclear. In addition, recent studies have shown some evidence for non-physical modes of reproductive regulation such as a possible connection between juvenile hormone and CHCs, alpha females having certain distinguishing features in their CHC profiles etc. This has led to the belief that perhaps queens do use cuticular hydrocarbons as a means to regulate reproduction in workers in some species of *Polistine* wasps (Kelstrup et al., 2015; Smith and Liebig, 2017; Dani and Turillazzi, 2018; da Silva et al., 2020; Tsuchida et al., 2020; Oi et al., 2021). Whether this is true also in the case of *R. cyathiformis* is not known and would have to be studied.

Linear hierarchy formation requires repeated interaction by aggressive individuals, and this was prevented on a nest with a despotic queen that suppressed the aggressive behavior of nestmates. The existence of pheromonally maintained hierarchies, perceived by wasps, but invisible to the human observer, remains an intriguing possibility (West-Eberhard 1986).

6.2. *R. marginata*

Unlike *R. cyathiformis*, the queens of *R. marginata* are meek and docile individuals who rarely participate in the dominance-subordinate interactions of the colony. Hence, she cannot possibly be regulating the reproduction of her workers by means of dominance behaviour. Nevertheless, since *R. marginata* queens maintain a complete reproductive monopoly in their colonies, worker reproduction is somehow suppressed. One hypothesis is that, as in the case of highly eusocial species, the queen may suppress worker reproduction or at least signal her presence and fertility using pheromones.

Experiments on *R. marginata* show that the queen

may be using a non-volatile pheromone to suppress worker reproduction or at least to signal her presence. Workers do not perceive the presence of the queen when separated from her by a wire mesh screen (Sumana et al., 2008). To address the question of how the workers might perceive the non-volatile pheromone of the queen, Bhadra et al. (2007) examined the possibilities of direct or indirect physical interactions between queens and workers. They found that the time required for the workers to perceive the absence of the queen if she used direct or indirect interaction would be much more than the observed 30 minutes that the workers take to detect the queen's absence. This led them to conclude that the queen might be depositing her pheromone by rubbing her abdomen on the nest. Additional experiments showed that the Dufour's gland in the queen contains the pheromone that the queen uses to communicate to the workers in the colony. The Dufour's glands of the queens and workers contain at least 30 detectable hydrocarbons. Although no compound seems to be unique to the queens, the relative proportions of the different compounds vary significantly between queens and workers. Discriminant function analysis separates the queen and workers into two distinct groups based on the composition of their Dufour's gland (Bhadra et al., 2010; Mitra et al., 2011) (Fig. 20), although there is no difference in the morphology of the gland (Mitra and Gadagkar, 2012). A bioassay showed that the PQ reduced her aggression when a macerate of the queen's Dufour's gland was applied to the nest surface in the absence of the queen. The idea that workers perceive the queen pheromone that she applies to the nest surface is reinforced by experiments in which queens and workers are observed in the absence of a nest. In such a situation, the queen rubs her abdomen on the walls of the cage. But the workers nevertheless attack the queen presumably because the pheromone concentration is now reduced (Saha et al., 2012). The use of pheromones by *R. marginata* queens is reminiscent of highly eusocial species where the queens use chemical means to regulate worker reproduction (Winston and Slessor, 1992; Keller and Nonacs, 1993; Katzav-Gozansky et al., 2002; D'Ettorre et al., 2004; Monnin, 2006; Chapuisat, 2014; Van Oystaeyen et al., 2014) and unlike primitively eusocial wasps who predominantly use dominance behaviour. However, recent studies in primitively eusocial wasps indicate that perhaps they also use chemical means to regulate reproduction, suggesting that non-physical means of reproductive regulation are not limited to highly eusocial species.

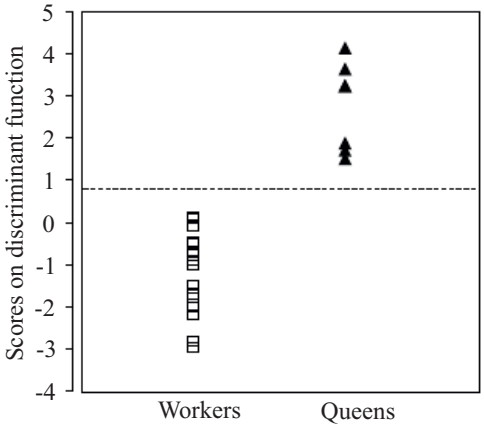


Fig. 20. Scores on discriminant function analysis for gas chromatographic analyses of Dufour's glands of *Ropalidia marginata* queens and workers (n. queens=7, n. workers=16, Wilk's λ=0.214, P<0.001, classification analysis: 100% correct classification) (Reproduced with permission from Bhadra et al., 2010)

7. Regulation of non-reproductive division of labour - age polyethism

7.1. *R. cyathiformis*

As we have seen above, there is a rudimentary age polyethism in *R. cyathiformis,* but the tasks that individuals perform are a function of their absolute age and not relative to the ages of the other members of the colony (Unnikrishnan and Gadagkar, 2020). Such dependence on absolute age is bound to make the system much less flexible than a relative age-based division of labour. Age polyethism based on relative age allows the colony to respond to any changes in its age demography. In the event of the loss of workers of a particular age group due to predation or harsh environmental conditions, the colony can compensate for this by employing the remaining individuals based on their relative age. Such flexibility is not possible when an individual performs the task depending on its absolute age and not relative age in relation to the other individuals present in the colony. However, since there is no clear-cut partitioning of intranidal and extranidal tasks, indicating that individuals perform both simultaneously (Unnikrishnan and Gadagkar, 2020). Therefore, in the case of *R. cyathiformis*, although age polyethism is much less flexible, the colony is resistant to any demographic changes because individuals simultaneously perform all tasks.

7.2. *R. marginata*

Unlike in *R. cyathiformis*, individuals of *R. marginata* modulate the relative frequency of task performance with respect to their relative age instead of their absolute age (section 4.2). A relative age-based

polyethism, unlike an absolute age-based polyethism, allows individuals to compensate for any changes in the colony age demography making the age polyethism a highly flexible one. This flexibility in age polyethism was explicitly shown in experiments that changed the colony demography and observed changes in age polyethism. When colonies consisting of only very young individuals were studied, precocious foragers were observed as individuals started foraging at a much younger age than they would have in a colony with older individuals present (Naug and Gadagkar, 1998b). Interestingly, when this experiment's converse was performed, with colonies where young individuals were removed, it did not result in over-aged nurses nursing at very old ages; instead, it resulted in hard-worked nurses nursing at abnormally high rates (Agrahari and Gadagkar, 2004). To better explain the flexibility in age polyethism in *R. marginata,* an activator-inhibitor model was developed (Naug and Gadagkar, 1999). The model clearly shows how increasing task demands can be met by a decrease in the ages of first performance and an increase in the proportions of individuals engaged in various tasks. When the role of an important regulator of age polyethism from studies on honey bees, the juvenile hormone (JH), was studied, it revealed that JH increased ovarian development in *R. marginata* workers but did not play a role in regulating age polyethism (Agrahari and Gadagkar, 2003). In *R. marginata,* as there is a strict separation of intranidal and extranidal tasks, a flexible age polyethism will allow the colony to adjust and compensate for any changes in the colony demography.

8. Regulation of non-reproductive division of labour - foraging and feeding larva

8.1. *R. cyathiformis*

Since the queen in *R. cyathiformis* is a behaviourally dominant individual (see section 2.1), and she seems to suppress worker reproduction using dominance behaviour (see section 6.1), she might also be expected to regulate the non-reproductive activities of the workers, such as foraging and feeding of the larvae. However, we saw in section 5.1 that even when the queen was removed, foraging and feeding of the larvae continued unabated. In contrast, the absence of the queen results in lower activity levels of the workers in some other species such as *Polistes instabilis*, *P. dominulus* (Jha et al., 2006) and *P. versicolor* (Souza and Prezoto, 2012) but not in the case of *P. fuscatus* (Reeve and Gamboa, 1983, 1987). How are their activities regulated if the queen is unnecessary for workers to forage and feed the larvae in *R. cyathiformis*? In other words, how do the workers know how much to forage and feed the larvae? For the workers to regulate their foraging and feed the larvae, they need information about the colony's hunger level. How do they get this information? It is conceivable that information about the colony's hunger level can be obtained while feeding the larvae. We find a significant positive correlation between the rates at which workers forage and feed larvae (Unnikrishnan and Gadagkar, 2021), implying that the same individuals who forage for food feed the larvae well (Fig. 21). Thus, foragers can directly obtain information about the colony's hunger levels while feeding larvae and regulating their own foraging rates. Based on the aggressive behaviour of the queen Kardile and Gadagkar (2003) had previously speculated that the queen may act as the central pacemaker in *R. cyathiformis*. And based on the finding that the PQ increased her levels of feeding unloading the foragers and feeding the larvae, they speculated that role of the pacemaker passes on from the queen to the PQ after queen removal. This interpretation would be reasonable if the queen was indeed a pacemaker in queen-right colonies. However, we now find that the levels of dominance received by the workers do not predict their foraging levels. On the other hand, we find a weak, but significant positive correlation between the rates at which individual wasps bring food and their own rates of feeding the larvae. We therefore speculate that foragers in this species may regulate their own foraging levels by obtaining direct information about larval hunger levels through their significant contribution to larval feeding.

8.2. *R. marginata*

Similar to *R. cyathiformis*, the absence of the queen results in no difference in the rates of foraging

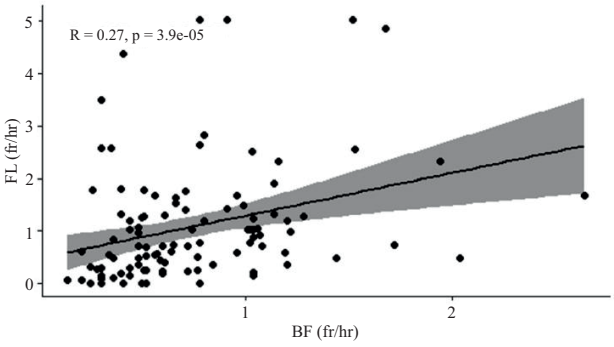


Fig. 21. Visualisation of correlation between Bring Food (BF) behaviour and Feed Larva (FL) behaviour. Kendall's correlation coefficient, $\tau = 0.27$, $P < 0.0001$. Data points represent fr/ hr of FL for each forager for that particular freq/hr of BF for 23 colonies (Reproduced with permission from Unnikrishnan and Gadagkar, 2021)

and feeding the larvae by the rest of the workers in *R. marginata*, indicating that the queen does not regulate the non-reproductive tasks of the colony. But in contrast to *R. cyathifrmis*, in *R. marginata*, there is a positive correlation between dominance behaviour received and bring food behaviour with several acts of dominance behaviour directed at foragers (Premnath et al., 1995). To better understand how dominance behaviour is used to regulate foraging, colonies kept inside wooden boxes in the lab were observed after excess feeding them. On the first day, unmanipulated colonies were observed, and all behaviours, including all dominance behaviour shown and received, were recorded. On the second day, the colony was fed in excess, with individuals being hand-fed with food in addition to their normal foraging. Interestingly when the demand for food was less in the colony, there were fewer acts of dominance-subordinate behaviour (Bruyndonckx et al., 2006) (Fig. 22). Hence this experiment suggests that the dominant-subordinate interactions are used for the regulation of foraging. To further understand this, the converse of this experiment was performed, where instead of excess-feeding the colony, the colony was starved on the second day. It was observed that when the colony was starved, the individuals that received the most aggression from other workers were the foragers (who were identified on day one as having brought food). And on the third day, once the colony was again fed, this aggression towards foragers came down (Lamba et al., 2008) *Ropalidia marginata* queens are strikingly docile and non-aggressive individuals, never at the top of the behavioural dominance hierarchy of their colonies. Nevertheless, these queens are completely successful at suppressing worker reproduction, suggesting that they do not use aggression but employ some other mechanism (e.g. pheromones. This aggression was directed towards the foragers (Fig. 23). Hence this experiment further confirms the role of dominance behaviour in the regulation of foraging by indicating that intranidal workers use dominance behaviour to communicate the colony hunger status to the extranidal workers or foragers. In *R. marginata*, workers regulate foraging among themselves in a decentralized manner of work regulation with intranidal workers communicating colony hunger levels using dominance behaviour to the extranidal workers or foragers.

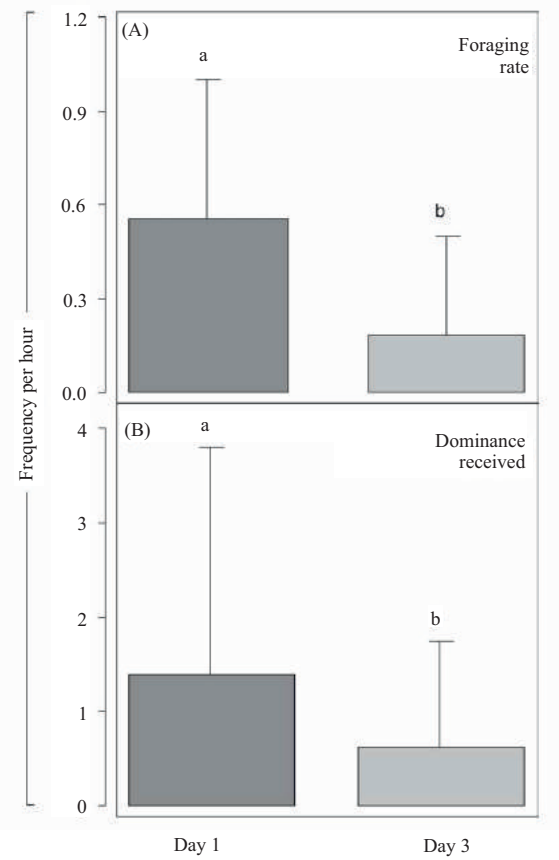


Fig. 22. Comparison of: (A) foraging rate; (B) dominance received by the 49 active foragers on day 1 (un-manipulated nests) and day 3 (one day after excess feeding). Bars shown are the means and S.D.'s for the 49 foragers. Both variables on day 1 are significantly greater than the corresponding variables on day 3 (two tailed Wilcoxon matched-pairs, signed-ranked tests; $p < 0.05$) (Reproduced with permission from Bruyndockk et al., 2006)

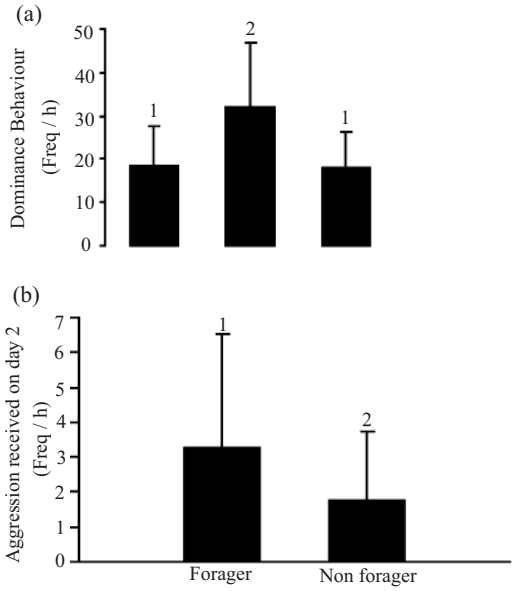


Fig. 23. (A) Mean and SD of a frequency per hour of dominance behaviour, on days 1, 2 and 3. (B) Mean and SD of frequencies per hour of aggression received on day 2 by foragers and non-foragers of day 1. Different numbers indicate a significant difference between the bars (Reproduced with permission from Lamba et al., 2008)

9. Insights into the evolution of eusociality

It is clear from the above comparisons that *R. cyathiformis* behaves as expected of a typical primitively eusocial wasp, with a behaviourally dominant queen, dominance-based queen succession, weak and rigid age polyethism, dominance-based reproductive regulation and lack of clear-cut separation of intranidal and extranidal task performance schedules. *R. marginata,* on the other hand, does not always behave as expected of a typical primitively eusocial wasp and displays some unusual traits. Queens are meek and docile, queen succession is not based on dominance behaviour, there is a well-developed and flexible age polyethism, clear-cut separation of intranidal and extranidal task performance schedules and the use of pheromones for reproductive regulation. Most of these traits displayed by *R. marginata* are reminiscent of highly eusocial species. However, *R. marginata* is also quite different from a typical highly eusocial species because it does not display queen-worker dimorphism, colony sizes are relatively small (rarely exceeding 100 individuals), and cast differentiation is largely post-imaginal (although there is some pre-imaginal caste bias (Gadagkar et al., 1988, 1990). *R. marginata*, being somewhat different both from primitively eusocial as well as highly eusocial species, may be thought of as being intermediate (Kardile and Gadagkar 2003; Gadagkar 2001; Deshpande et al. 2006; Saha et al. 2012; Chakraborty et al. 2018). We do not claim that eusociality necessarily involves a linear trajectory starting from a typical primitively eusocial species such as *R. cyathiformis,* passing through an intermediate stage such as *R. marginata* and finally evolving into a typical highly eusocial species such as the honey bees. That eusociality could have evolved by different trajectories and that we should not commit ourselves to a single, "ladder-like" trajectory has recently been emphasized by Linksvayer and Johnson (2019). Rather than suggest a trajectory going from *R. cyathiformis* through *R. marginata* to honey bees, we make the following very different point. Our comparison of *R. cyathiformis* and *R. marginata* concerning what is known about typical primitively and highly eusocial species suggests that traits such as pheromonal regulation of reproduction and age polyethism can evolve even without queen-worker dimorphism and increase in colony size. In other words, pheromonal regulation of reproduction and age polyethism could, at least sometimes, be the cause and queen-worker dimorphism and large colony size be the effect, rather than the other way around.

We are conscious that our conclusions are placed on comparing only two species, *R. cyathiformis and R. marginata,* and that one may arrive at different conclusions by studying other primitively eusocial species. We believe that detailed studies of even a small number of species could be very valuable (Goymann and Schwabl, 2021) and we, therefore, call for more detailed studies of many different primitively eusocial species, both in the tropics and in the temperate regions. We emphasize that we need careful documentation of phenomena such as queen-worker dimorphism, queen succession, the role of dominance behaviour, regulation of reproduction, age polyethism and task partitioning in a large number of species. Comparative analyses of the kind we are advocating are served better by behavioural data on many species rather than uncovering detailed physiological and molecular mechanisms of behaviour. In any case, the latter can only be attempted in a small number of species by relatively well-endowed research groups. Behavioural data of the kind we are seeking can be relatively easily obtained by large numbers of researchers, in a large number of habitats, without the need for expensive laboratory and technology. In addition to calling for such behavioural studies, preferably by researchers sympatric with their species of study, we also plead that such behavioural studies should not be considered less fashionable and 'sexy' than studies that attempt to understand the molecular basis of behaviour (Gadagkar, 2019, 2021).

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A CHECKLIST OF INSECT PESTS OF ONION

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ABSTRACT

A checklist of onion insect pests is given herein with 149 species of eleven arthropod orders, viz., Coleoptera, Collembola, Dermaptera, Diptera, Orthoptera, Hemiptera, Hymenoptera, Thysanoptera and Lepidoptera and two of Acarina, such as Sarcoptiformes and Trombidiformes. This comprises 49 families of which 46 are insect and three of Acarina. Insect family, Noctuidae includes maximum genera (16), species (27) followed by the Aphididae (8), Agromyzidae (8), Thripidae (7), Anthomyiidae (6), Syrphidae (6), Acarididae (6), Pyralidae (5), Scarabaeidae (5), Tetranychidae (4), Muscidae (4), Fanniidae (4), Nitidulidae (3), Onychiuridae (3), Eriophyidae (2), and Simunthridae (1) are others observed. Diversity analysis revealed that foliage feeders are the dominant (69.1%) comprising of sucking pests (aphids and thrips), defoliators (cutworms and leaf eating caterpillars) and leaf miners. Bulb feeders account about 18.8% (flies, beetles, weevils, moths and mites), and root feeders represent 8.7%.

Key words: *Allium* sp., pests, insects, mites, foliage, feeders, sucking pest, defoliators, bulb feeders, root feeders, families, genera, species

Onion is one of the important bulb vegetables grown in India and other parts of the world. India ranks first in cultivation area and second in production (Kumar et al., 2015) and is cultivated about 1.3 million hectares area with the annual production of 22.40 mt (FAOSTAT, 2017). Onion is attacked by number of insect pests including thrips, maggots, cut worms, leaf miner, aphids, beetles, earwigs and mites at different stages of crop growth, including seedling, bulbing and blooming. In addition to direct damage, insect pest such as thrips *Thrips tabaci* Lindeman and aphids *Myzus persicae* (Sulzer) and *Aphis craccivora* C.L Koch acts as vector for deadly viral diseases such as iris yellow spot (Pozzer et al., 1999) and onion yellow dwarf virus (Mahmoud et al., 2008). Furthermore, some insect pests also injurious to harvested bulbs at storage which reduce the quality and export potential of the produce. Being a major consuming, exporting as well as importing commodity to fulfill the domestic requirement, knowledge and information of insect pests is vital for pest management planning. Hence, an updated list of insect pests attacking onion at field as well as storage is necessary for devising preventive and effective IPM strategies. Therefore, in this report, a detailed list of onion insect pests is presented.

Checklist

The present compilation provides an up-to date comprehensive checklist of onion pests with literature compiled and condensed in the form of a checklist. Insect pests were grouped according to their taxonomic level as well as their feed habits. The insect feeding stage, parts fed, distribution and corresponding references have been compiled and included. A total of 149 pests, including eight insects and two acarines, 49 arthropod families and 97 insect genera are known worldwide (Table 1). Order and family wise and genera and species are provided. Among these, a maximum number of species fall under Noctuidae (16 genera and 27 species), followed by mites, Aphididae, Agromyzidae, Anthomyiidae and Syrphidae, Muscidae and Thripidae and Elateridae, and Acrididae and Scarabaeidae (Fig. 1). Others with one or few genus and species are Chrysomelidae, Curculionidae, Anobiidae, Meloidae, Furficulidae, Ephydridae, Heliomyzidae, Sciaridae, Ulidiidae, Aleyrodidae, Delphacidae, Pentatomidae, Geometridae, Pieridae, Tineidae, Sminthuridae, and Gryllotalpidae. Literature revealed that foliage eating insects (stem / pseudo stem / leaf) was dominant (69.1%) followed by bulb feeders (18.8%), root feeders (8.7%), umbel / flower feeders (2.7%) and seed feeders (0.7%) (Fig. 2). Onion thrips, *Thrips tabaci* Lindeman and western flower thrips, *Frankliniella occidentalis* (Pergande) are major sucking pests causing economic damage worldwide. Thrips act as vector for deadly Irish Yellow Spot Virus in both the onion seed crop and the bulb crop. Beside aphids *Myzus* sp and Neotoxoptera sp were dominant sucking pests of onion. Onion maggot, *Delia antiqua* is a major dipteran pest of onion, particularly in the cooler regions. Cutworm *Spodoptera*

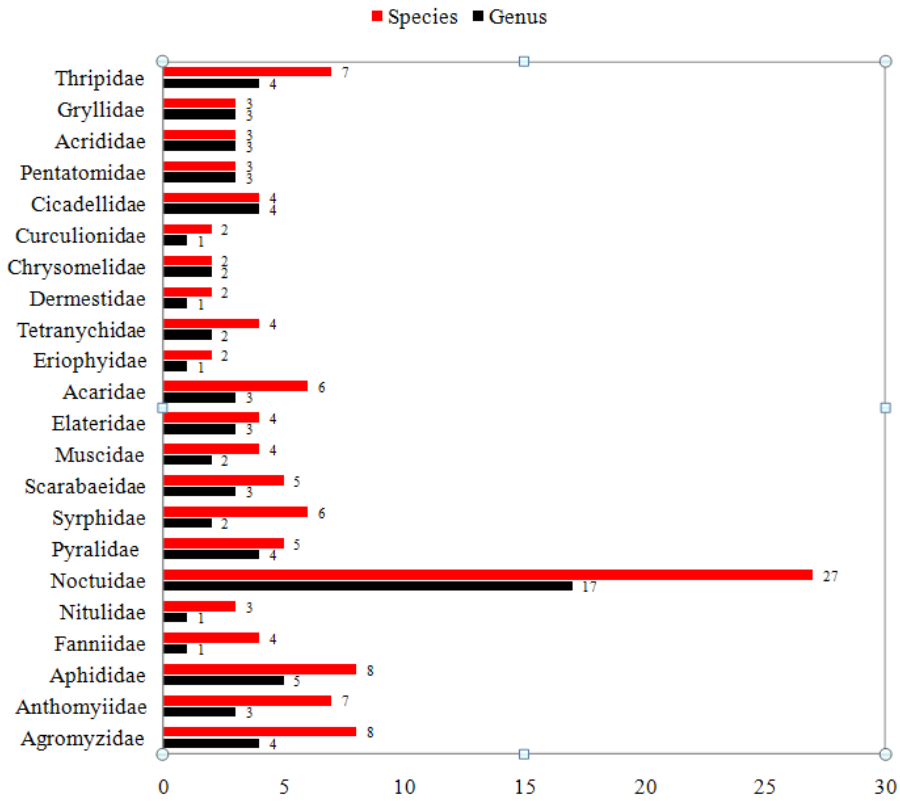


Fig. 1. Major insect families associated with onion

sp, *Euxoa* sp, *Peridroma* sp and *Agrotis* sp are also predominant lepidopterans infesting onion. Dried fruit beetle *Carpophilus* sp., cigarette beetle, *Lasioderma serricorne* (F.), warehouse moths, *Plodia interpunctella* (Hubner), *Ephestia cautella* (Walker) and bulb mite, *Rhizoglyphus* sp are important pests at storage causing economic damage. Apterygote insects, Collembola, springtails, *Onychiurus* sp. have been also documented as root feeders in onion.

Kaundal (2018) had earlier provided a global and Indian scenario of insect pests infesting onions. He listed 77 insect and seven mite species from 10 orders and 31 families are known worldwide. Of these, Lepidopterans are the most abundant, followed by dipterans, hemipterans and coleopterans. Pests

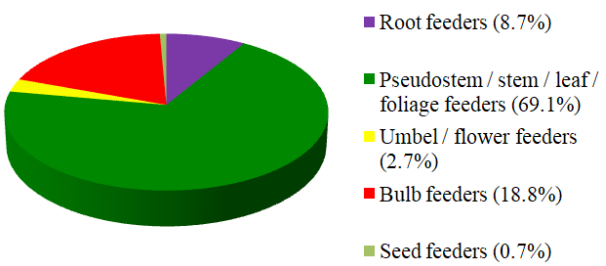


Fig. 2. Functional diversity of insect pests of onion

including onion thrips *Thrips tabaci* Lindeman, onion maggot *Delia anitqua* (Meign), beet army worm, *Spodoptera exigua* Hubner, head borer *Helicoverpa armigera* (Hubner), golden wing moth *Trichoplusia orichalcea* F., cut worm *Agrotis* sp., bulb mite *Rhizoglyphus robini* (Claparede), and red spider mite, *Tetranychus cinnabarinus* (Boisduval) are the major pests. In India, 25 insect and four mite species from eight orders and 13 families have been documented, with ten of them classified as pests (Kaundal, 2018; Kaundal and Sood., 2021). *Altica* sp., *Chromatomyia horticola* (Goureau), *Euconocephalus* sp., *Gryllus* sp., *H. armigera* (Hubner), *Melolontha furicicauda* Ancey, *Nezara viridula* L., *Spodoptera litura* (F.), *T. tabaci* and *T. orichalacea* are some pests; *T. tabaci* is the major pest, followed by *D. antiqua,* whereas, *H. armigera, A. ipsilon,* and *S. litura* are minor pests (Gupta et al., 1991). Bulb fly, *Atherigona orientalis* and dried fruit beetle *Carpophilus obsoletus* Erichson have been found in stored onion bulbs. *D. antiqua* is a problem in cold arid Ladak region of India (Gupta et al., 2019); *T. tabaci*, *D. antiqua*, *R. robini*, eriophyid mite *Aceria tulipae* Keifer and *T. cinnabarinus* are pests of national significance; while *H. armigera*, *A. ipsilon*, and *S. litura* are pests of regional significance (Satyagopal et al., 2014). In addition, the beet army

Table 1. Checklist of insect pests of onion

S. No.	Order/ Family/ Common Name/ Scientific Name	Distribution	Nature of feeding	Damaging stage	Reference
	Coleoptera				
	Chrysomelidae				
1	Onion beetle, *Lilioceris merdigera* (L., 1758)	Poland, Iran	Seedlings	Larva / grub	Luczak (1994); Szwejda and Wrzodak (2009)
2	Flea beetle *Altica* sp	India	Leaf	Larva / grub	Kaundal and Sood (2021)
	Curculionidae				
3	Weevil, *Ceuthorrhynchus jakovlevi* Schultze, 1902	Poland	Seedlings	Larva / grub	Ruszkowska (1952); Osmolowski (1980); Szwejda and Wrzodak (2009)
4	*Ceuthorrhynchus suturalis* F., 1775	Poland	Seedlings	Larva / grub	Jerzy and Robert (2009)
	Dermestidae				
5	*Anthrenus jordanicus* Pic, 1934	India	Bulb	Grub and Adult	Chandel et al. (2016)
6	*Anthrenus ocenicus* Fauvel, 1903	India	Bulb	Grub and Adult	Chandel et al. (2016)
	Elateridae				
7	Wireworm, *Agriotes lineatus* (L., 1767)	Poland	Seedlings	Larva / grub	Szwejda and Wrzodak (2009)
8	Wheat wireworm, *Agriotes mancus* (Say, 1823)	USA	Root	Grub and Adult	Chittenden (1913)
9	Wireworm, *Anthos* sp	Poland	Seedlings	Larva / grub	Szwejda and Wrzodak (2009)
	Nitidulidae				
10	Dried fruit beetle, *Carpophilus hemipterus* (L., 1758)	Egypt	Bulb	Nymph and adult	Zaazou et al. (1960); Sabra et al. (2011); Ibrahim et al. (1970); Abu-Hashish (1998)
11	Dried fruit beetle, *Carpophilus immaculatus* Lucas, 1849	Egypt	Bulb	Nymph and adult	Sabra et al. (2011)
12	*Dried fruit beetle, Carpophilus obsolutus* Erichson, 1843	India, Egypt	Bulb	Nymph and adult	Gupta et al. (1991)
	Anobiidae				
13	Cigarette beetle, *Lasioderma serricorne* (F., 1972)	Egypt	Bulb, Seed	Nymph and adult	Dimetry et al. (1978); Verma (2011)
	Scarabaeidae				
14	Cockchafer, *Melolontha melolontha* (L., 1758)	Poland	Seedlings	Larva / grub	Szwejda and Wrzodak (2009)
15	Cockchafer, *Melolontha hippocastani* F., 1801	Poland	Seedlings	Larva / grub	Szwejda and Wrzodak (2009)
16	Cockchafer, *Melolontha furicicauda* Ancey	India	Seedlings	Larva / grub	Kaundal (2018)
17	*Phyllophaga* sp	USA	Seedlings	Larva / grub	Hall et al. (2011)
18	Hairy beetle, *Tropinota squalidae* (Scopoli, 1783)	Egypt	Leaf/ Flower	Adult	Elbolok et al. (1990)
	Dynastidae				
19	Potato scrabid, *Pentodon bispinosus* Kust, 1852	Egypt	Leaf	Adult	Elbolok et al. (1990)
	Meloidae				
20	Nuttall blister beetle, *Lytta nuttalli* Say, 1824	US	Leaf	Adults	Capinera (2001)
	Collembola				
	Onychiuridae				
21	Springtails, *Onychirus armatus* (Tulberg, 1869)	Tasmania	Root	Nymph and adult	Ireson (1990); Ireson (1993)

(contd.)

22	Springtails, *Onychirus fimatarius* Stach, 1934	Tasmania	Root	Nymph and adult	Ireson (1990)
23	Springtails, *Onychirus ambulans* (L., 1758)	Tasmania	Root	Nymph and adult	Ireson (1990)
	Entomobryidae				
24	Springtails, *Entomobrya unostrigata* Stach, 1930 Bourletiellidae	USA	Root	Nymph and adult	Scott (1964)
25	Garden springe tails*, Bourletiella hortensis* (Fitch, 1863)	Tasmania	Root	Nymph and adult	Ireson (1990); Mills (1930)
	Sminthuridae				
26	Garden springtails, *Siminithurus viridis* (L., 1758)	New Zealand	Root	Nymph and adult	Munro (2007)
	Dermaptera				
	Furficulidae				
27	Ringlegged earwig, *Euborellia annulipes* (*Lucas*, 1847)	India	Bulb, Seedlings	Nymph and adult	Hill (1983); Chandel et al. (2016)
	Diptera				
	Agromyzidae				
28	Allium leaf miner*, Phytomyza gymnostoma* Loew, 1858	UK, Denmark, USA	Leaf	Larva	Coman and Rosca (2011); Spencer (1976); Simoglou et al. (2008); Collin and Loe (2005); USDA (2016); Barringer et al. (2018)
29	Vegetable leaf miner*, Liriomyza huidobrensis* (Blanchard, 1926)	Chile, Peru, China, Columbia, Costa Rica, Germany, Guatemala, Indonesia, Kenya, Philippines, Spain, Taiwan, Vietnam	Leaf	Larva	Mujica et al. (2011); Weintraub et al. (2017); Andersen et al. (2008)
30	Vegetable leaf miner, *Liriomyza sativae* Blanchard, 1938	Hawaii, North America, Vietnam	Leaf	Larva	Carolina and Johnson (1992); Andersen et al. (2008); Chen et.al (2003)
31	American serpentine leaf miner, *Liriomyza trifolii* (*Burgess,* 1880)	Philippines, Hawaii, Vietnam	Leaf	Larva	Stegmaier (1966); Arida et al. (2013); Ronald and Jayma (2007); Andersen et al. (2008)
32	*Liriomyza chinensis* (Kato, 1949)	China, Japan, Malaysia, Singapore, Thailand, Korea Vietnam and Taiwan	Leaf	Larva	Spencer, (1973); Chen et al. (2003); Hwang and Moon (1995); Andersen et al. (2002); Shiao (2004); Uneo (2006); Chen et.al (2003)
33	Pea leaf miner, *Chromatomyia horticola* Goureau, 1851	Poland, Croatia, India, Vientnam	Leaf	Larva	Gupta et al. (1985); Szwezda and Wrzodac (2009); Mesic and Barcic (2004); Andersen et al. (2008); Kaundal and Sood (2021)
34	Onion leaf miner, *Liriomyza cepae* (Hering, 1927)	Europe, Malaya, China, Japan	Leaf	Larva	Sasakawa (1961)
35	Turnip leaf miner, *Scaptomyza flava* Fallen, 1823	New Zealand	Leaf	Larva	Martin (2004)
	Anthomyiidae				
36	Onion fly, *Delia antiqua* (Meigen, 1826)	Asia, Africa, North and South America, Europe	Bulb, Pseudo-stem	Maggot	Wang and Xiang (2004); Rozkosny (1997); Griffiths (1993); Pont (1974); Henning (1974)

(contd.)

37	Been fly, *Delia platura* (Meigen, 1826)	Australia Europe north and South America, North Africa, Japan, India, New Zealand	Bulb, Pseudo-stem	Maggot	Ellis and Scatcherd (2007); Hamilton and Toffolon (1986); CIE (1985); Chaudhary et al. (1987); Idinger (1995); Pont and Ackland (1980); Pont (1974); Munro (2007)
38	Been fly, *Delia florilega* (Zetterstedt, 1845)	Australia; Europe North and South America, North Africa, Japan, India, New Zealand	Bulb, Pseudo-stem	Maggot	Ellis and Scatcherd (2007)
39	Onion maggot, *Delia alliria* Fonseca, 1965	Egypt	Bulb	Maggot	Elbolok et al. (1990); Awadalla et al. (2011)
40	Root maggot, *Pegomya cepetorum* Meade, 1883	USA	Bulb	Maggot	Chittenden (1913)
41	Seed corn maggot, *Pegomya fusciceps (Zetterstedt, 1845)*	USA	Roots/ Stalks	Maggot	Chittenden (1913)
	Drosophilidae				
42	Fruit fly, *Drosophila busckii* Conquillett, 1901	USA	Bulb	Larva	Merrill and Hutson (1953)
43	Leaf mining fly, *Scaptomyza flava* Fallen (1823)	New Zealand	Leaf	Larva	Munro (2007)
	Ephydridae				
44	Rice leaf miner, *Hydrellia griseola* (Fallen, 1823)	Florida, (USA); USSR	Leaf	Larva	Hesler (1995); Isaev (1931)
	Fanniidae				
45	Lesser housefly, *Fannia canicularis* (L., 1761)	Poland, USA	Bulb	Larva	Szwejda and Wrzodak (2009); Merrill and Hutson (1953)
46	Latrine fly, *Fannia scalaris* (F., 1794)				
47	*Fannia leucosticta* (Meigen, 1838)				
48	*Fannia manicata* (Meigen, 1826)				
	Heleomyzidae				
49	Garlic fly, *Suillia lurida* Meigen, 1830	Poland, Iran	Bulb	Larva	Szwejda and Wrzodak (2009)
	Ortalidae				
50	Barred winged onion fly, *Chatopsis anea* (Wiedemman,1830)	USA, Canada	Root	Maggot	Chittenden (1913)
	Ulidiidae				
51	Black onion fly, *Tritoxa flexa* (Wiedemman, 1830)	USA, India	Root	Maggot	Chittenden (1913); Chandel et al. (2016)
52	Picture winged fly, *Chaetopsis massyla* (Walker, 1849)	Canada	Bulb	Larva	Merrill (1951)
	Muscidae				
53	Pepper fruit fly, *Atherigona orientalis* Schiner, 1868	Egypt, India	Bulb	Maggot	Mahmoud (2008); Gupta et al. (1991); Sabra et al. (2011);
54	False stable fly, *Muscina levida* (Harris, 1780)	Brazil, Poland, USA	Rotten bulb	Maggot	Merrill and Hutson (1953); Szwejda and Wrzodak (2009)
55	Fly, *Muscina assimilis* Fallen, 1823	Poland, USA	Bulb	Maggot	Carvalho et al. (2005); Merrill and Hutson (1953); Szwejda and Wrzodak (2009)
56	Fly, *Muscina stabulans* Fallen, 1817	Poland, USA	Bulb	Maggot	Carvalho et al. (2005); Merrill and Hutson (1953); Szwejda and Wrzodak (2009)

(contd.)

	Sciaridae				
57	Dark winged fungus gnat, *Bradysia odoriphaga* Yang and Zhang, 1985	China	Root, Bulb, Pseudo-stem	Larva	Yang and Zhang (1985); Arimoto et al. (2018)
	Syrphidae				
58	Onion bulb fly, *Eumerus amoenus* Loew, 1848	Egypt	Bulb	Maggot	El-Sherif and Mahmoud (2008); Haydar and El-Sherif (1987); Sabra et al. (2011)
59	Lesser bulb fly, *Eumerus strigatus* (Fallen, 1817)	United States, New Zealand, England; Poland, Australia, Egypt	Bulb	Maggot	Collin (1920); Braodbet (1925); Wilcox (1926); Doane (1983); Hodson (1927); Bankowska (1980)
60	Lesser bulb fly, *Eumerus tuberculatus* Rondani, 1857	England, Poland, Florida (USA)	Bulb	Maggot	Collin (1920); Bankowska (1980)
61	Lesser bulb fly, *Eumerus narcissi* Smith, 1928	Florida (USA), Oregon, UK	Bulb	Maggot	Wilcox (1926); Hodson (1932)
62	Onion bulb fly, *Eumerus vestitus* Bez	Egypt	Bulb	Maggot	Elbolok et al. (1990)
63	Onion bulb fly, *Syrrita spinigera* Lw	Egypt	Leaf	Adult	Elbolok et al. (1990)
	Hemiptera				
	Aphididae				
64	Shallot aphid, *Myzus ascalonicus* Doncaster, 1946	Europe, New Zealand	Leaf	Nymph and adult	Munro (2007)
65	Mustard aphid, *Lipaphis erysimi* (Kaltenbach, 1843)	Europe; Barbados	Leaf	Nymph and adult	Hill (1983); Braithwaite (1983)
66	Shallot aphid, *Myzus persicae* (Sulzer, 1776)	Europe	Leaf	Nymph and adult	Hill (1983)
67	*Black onion aphid, Neotoxoptera formosana (Takahashi, 1921)*	Europe, Japan, New Zealand	Leaf	Nymph and adult	Hori (2007); Piron (2010); Munro (2007)
68	Violet aphid*, Neotoxoptera violae (Pergande, 1900)*	Italy, Netherland, France	Leaf	Nymphs and adult	Barbagallo and Ciampolini (2000); Piron (2010)
69	Marigold aphid*, Neotoxoptera oliveri (Essig, 1935)*	New Zealand	Leaf	Nymphs and adult	Munro (2007)
70	Cotton aphid, *Aphis gossypii* Glover, 1877	Egypt	Leaf	Nymph and adult	Awadalla et al. (2017)
71	Foxglove aphid, *Aulacorthum solani* (Kaltenbach, 1843)	Europe	Leaf	Nymph and adult	Hill (1983)
	Aleyrodidae				
72	Cotton and tomato white fly, *Bemisia tabaci* (Gennadius, 1889)	Egypt	Leaf	Nymph and adult	Elbolok et al. (1990)
	Cicadellidae				
73	Leaf hopper, *Empoasca decipines* Paoli, 1930	Egypt	Leaf	Nymph and adult	Elbolok et al. (1990)
74	Leaf hopper, *Balclutha hortensis* Lindberg, 1954	Egypt	Leaf	Nymph and adult	Elbolok et al. (1990)
75	Jassid, *Amrasca biguttula biguttula* Ishida, 1912	India	Leaf	Nymph and adult	Gupta et al. (2016); Kumar et al. (2017)
76	Ash leaf hopper, *Macrosteles fascifrons* (Stal, 1858)	India	Leaf	Nymph and adult	Chandel et al. (2016)
	Delphacidae				
77	Plant hopper, *Sogatella vibix* (Haupt, 1927)	Egypt	Leaf	Nymph and adult	Elbolok et al. (1990)
	Pentatomidae				
78	Southern green stink bug, *Nezara viridula* (L., 1758)	Egypt	Leaf	Nymph and adult	Elbolok et al. (1990)
79	Shield bug, *Carpocoris fuscipinus* Boh, 1851	Poland	Leaf		Jerzy and Robert (2019)

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80	Shield bug, *Dolycoris baccarum* L., 1785	Poland	Leaf	Nymph and adult	Jerzy and Robert (2019)
	Triozidae				
81	Psyllid, *Trioza brassicae* Vasiliev, 1922	Poland	Leaf	Nymph and adult	Jerzy and Robert (2019)
	Hymenoptera				
	Formicidae				
82	Red ant, *Solenopsis* sp	Ethiopia	Bulb	Adult	Haile et al (2016)
	Lepidoptera				
	Acrolepiidae				
83	Asiatic onion leaf miner, *Acrolepiopsis sapporensis* Matsumura, 1931	Korea, Japan	Leaf	Larva	Park (2015); Rabinowitch and Brewster (1990)
84	Leek moth, *Acrolepiopsis assectella* (Zeller, 1839)	North America	Leaf, bulb	Larva	Olmstead and Shelton (2012); Harmatha et. al. (1987); Mason et al. (2011)
	Geometridae				
85	The loop worm, *Gymnoscelis pumilata* (Hubner, 1813)	Egypt	Leaf	Larva	Elbolok et al. (1990)
	Cosmopterygidae				
86	Pyroderces moth, *Pyroderces simplex* Wlsm 1891	Egypt	Leaf	Adult	Elbolok et al. (1990)
	Noctuidae				
87	Beet army worm, *Spodoptera exigua* (Hubner, 1808)	Netherlands, Japan, Korea, North America, Egypt, Philippines, Vietnam, India	Leaf / Umbel	Larva	Wakamura et al. (1989); Ueno (2006); Ueno (2015); Zheng et al. (2000); Heppner (1998); Arida et al. (2004); Rao (1998); Elbolok et al. (1990); Kang et al. (2008); Park and Goh (1992); Soumia et al. (2020)
88	Cutworm, *Spodoptera litura* (F., 1775)	India, Philippines	Leaf / Umbel	Larva	Prasad and Shrisha (2009); Arulkumar et al (2017); Arida et al. (2004); Rao (1998)
89	Cotton leafworm, *Spodoptera littoralis* (Boisduval, 1833)	Barbados, Africa, Europe	Leaf	Larva	Brathwaite (1983); EFSA (2015); Elbolok et al. (1990)
90	Black cutworm, *Agrotis ipsilon* (Hufnagel, 1766)	Mozambique, India, Egypt Canada, South America	Leaf, root, pseudo-stem	Larva	Sathyagopal et al. (2014); Sulvai et al. (2016); Elbolok et al. (1990); Rabinowitch and Brewster (1990);
91	Pale-Sided Cutworms, *Agrotis malefida* Guenee, 1852	Brazil	Leaf, root, pseudo-stem		Specht et al. (2013)
92	Old word boll worm, *Helicoverpa armigera* (Hubner, 1809)	India, Pakistan, Burkina Faso	Leaf and umbel	Larva	Rai et al. (1979); Ahmed et al. (2019); Rubiya et al. (2019); Srinivas et al. (2007)
93	Green looper, *Chrysodeixis acuta* (Walker, 1858)	India	Leaf	Larva	Karuppaiah et al. (2019)
94	Semilooper, *Trichoplusia orichalcea* F., 1775	India	Leaf	Larva	Kaundal (2018)
95	Oriental tobacco budworm, *Heliothis assulta* Guenee, 1852	Papua NG, Australia	Leaf	Larva	Hill (1983); Cho and Boo (1988); Cork et al. (1992)
96	Large yellow under wing, *Noctua pronuba* L., 1758	North America	Seedlings/ leaf	Larva	Bechinski et al. (2009)
97	Turnip moth, *Agrotis segetum* (Denis &and Schiffermüller, 1775)	U.K	Leaf	Larva	Rabinowitch and Brewster (1990)
98	Leaf worm, *Copitarsia* spp	Chile, Peru	Leaf	Larva	Valasquez (1988); Venette and Gould (2006); Rabinowitch Brewster (1990)

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99	Turnip moth/yellow cutworm, *Scotia segetum* (Denis and Schiffermüller, 1775)	Denmark	Pseudo-stem / leaf	Larva	Esbjerg et al. (1995)
100	Garden dart moth, *Euxoa nigricans* (L., 1761)	Europe	Pseudo-stem, leaf, Seedling	Larva	Carter (1984); Rabinowitch Brewster (1990)
101	Whiteline dart moth, *Euxoa tritici* (L., 1761)	Europe	Seedling / leaf	Larva	Rabinowitch Brewster (1990)
102	Army worm, *Faronta* spp	Chile	Stem	Larva	Rabinowitch Brewster (1990)
103	Onion stem borer, *Hydraecia mongoliensis* (Urbahn, 1967)	Japan	Pseudo-stem / leaf	Larva	Saito (1984); Iinuma and Saito (1988)
104	Cabbage moth, *Mamestra brassicae* (L., 1758)	UK	Leaf	Larva	Rabinowitch Brewster (1990)
105	Variegated cutworm, *Peridroma saucia* (Hubner, 1808)	USA	Pseudo-stem / leaf	Larva	Rabinowitch Brewster (1990)
106	Variegated cutworm, *Peridroma margcmtosa* Haw.	USA	Pseudo-stem / leaf	Larva	Chittenden (1913)
107	Army cutworm, *Chorizagrotis auxiliaries* (Grote, 1873)	USA	Pseudo-stem / leaf	Larva	Whelan (1935)
108	Dark-sided cutworm, *Euxoa messoria* (Harris, 1841)	USA	Pseudo-stem / leaf	Larva	Chittenden (1913)
109	Striped Cutworm, *Euxoa tessellata* (Harris, 1841)	USA	Pseudo-stem / leaf	Larva	Chittenden (1913)
110	Dark-sided cutworm, *Euxoa punctigera* (Walker, 1865)	UAS	Stem / leaf	Larva	Chittenden (1913)
111	Clay-backed cutworm, *Feltia gladiaria* Morrison, 1875	USA	Pseudo-stem / leaf	Larva	USDA (1932)
112	Spotted cutworm, *Agrotis c-nigrum* L., 1758	USA	Pseudo-stem / leaf	Larva	Whelan (1935)
113	Moth, *Margarita stricticalis*	Poland	Pseudo-stem / leaf	Larva	Jerzy and Robert (2009)
	Pieridae				
114	Cabbage worm, *Pieris rapae* (L., 1758)	Egypt	Leaf	Adult	Elbolok et al. (1990)
	Pyralidae				
115	Christmas berry webworm, *Cryptoblabes gnidiella* (Milliere, 1867)	Egypt, US	Bulb		Rabinowitch Brewster (1990); Molet (2013)
116	Almond moth, *Ephestia cautella* (Walker, 1864)	India, Egypt	Bulb	Larva	Vengatesh and David (2001); Sabra et al. (2011)
117	Mediterranean flour moth, *Ephestia kuehniella* (Zeller, 1879)	Egypt	Bulb	Larva	Elbolok et al. (1990)
118	Indian meal moth, *Plodia interpunctella* (Hubner, 1813)	Egypt	Bulb	Larva	Elbolok et al. (1990); Ja HyunNa and Rayoo (2000)
119	Beet webworm, *Loxostege sticticalis* (L., 1761)	North America	Pseudo Stem / Leaf	Larva	Capinera (2001)
	Tineidae				
120	Garlic moth, *Cryptoblabes gnidiella* (Millere, 1867)	Egypt	Leaf / bulb	Larva	Elbolok et al. (1990)
	Toricidae				
121	Cyclamen tortrix, *Clepsis spectrana* (Treitschke, 1830)	UK	Leaf	Larva	Rabinowitch Brewster (1990)
122	Carnation tortrix, *Cacoecimorpha pronubana* (Hubner, 1799)	France	Leaf	Larva	Rabinowitch Brewster (1990)
	Orthoptera				
	Gryllotalpidae				
123	Common mole cricket, *Gryllotalpa gryllotalpa* (L., 1758)	Poland, Egypt	Leaf/ Pseudo-stem	Nymph and adult	Szwejda and Wrzodak (2009);

(contd.)

	Acrididae				
124	Egyptian locust, *Anacridium aegyptium* (L., 1758)	Egypt	Leaf	Adult	Elbolok et al. (1990)
125	Clover grass hopper, *Euprepocnemis plorans* (Charpentier, 1825)				
126	Grasshopper, *Attractomorpha sinensis* Bolivar, 1905	Pakistan	Leaf	Nymph and adult	Sarwar (2012)
	Gryllidae				
127	Black field cricket, *Liogryllus bimaculatus* (de Geer, 1773)	Egypt	Root, Bulbs	Nymph and adult	Elbolok et al. (1990)
128	Field cricket, *Brachytrupes portentosus* Licht, 1976	India	Root, Bulbs	Nymph and adult	Kaundal (2018)
129	Field cricket, *Gryllus pennsylvanicus* Burmeister, 1938	Pakistan	Root, Bulbs	Nymph and adult	Wagan et al. (2014)
	Tettigoniidae				
130	Grasshopper, *Eucanocephalus* sp	India	Leaf	Nymph and adult	Kaundal (2018)
	Thysanoptera				
	Thripidae				
131	Onion thrips, *Thrips tabaci* Lindeman, 1889	India, New Zealand, North America, Egypt, Australia	Leaf, bulb	Nymph and adult	Sakimura (1937); Passlow (1957); Lall and Singh (1968); Saxena (1975); Coudriet et al. (1979); Shelton et al. (1983); Toda and Morishita (2009); Gill et al. (2015); Srinivas et al. (2007); Diaz-Montano et al. (2010); Kendall and Capinera (1990); MacIntyre-Allen et al.(2005); Martin et al. (2006); Murai and Toda (2002); Pobożniak et al. (2016); Pozzer et al. (1999); Awadalla et al. (2017)
132	Western Flower Thrips, *Frankliniella occidentalis* Perganda, 1895	North America; Netherlands; China	Leaf,	Nymph and adult	Tommasini (2003); Mantel and Van de Vrie (1988); (Kirk and Terry (2003); Coviello, et al. (1995); Reitz et al. (2011)
133	Tobacco thrips, *Fancciniella fusca* (Hinds, 1902)	Japan, North America	Leaf , bulb	Nymph and adult	Shannon et al. (2008); Nakao et al. (2011)
134	Cabbage thrips, *Thrips angusticeps* Uzel, 1895	Europe	Leaf	Nymph and adult	Hurej et al. (2017)
135	Melon thrips, *Thrips palmi* Karny, 1925	India	Leaf	Nymph and adult	Sanjta and Chauhan (2015); Kaundal (2018)
136	Yellow flower thrips, *Thrips flavus* Schrank, 1776	India	Leaf	Nymph and adult	Sanjta and Chauhan (2015); Kaundal (2018)
137	Groundnut thrips, *Caliothrips indicus* Bagnall, 1913	India	leaf	Nymph and adult	Hill (1983)
			Non-insect pests		
	Sarcoptiforms				
	Acaridae				
138	Bulb mite, *Rhizoglyphus callae* Oudemans, 1924	Argentina	Leaf, bulb	Nymph and adult	Ho (2008)
139	Bulb mite, *Rhizoglyphus robini* Claparédè, 1869	Australia, New Zealand, USA, Japan, South America, UK, Egypt, China, Korea, India	Leaf, bulb	Nymph and adult	Hai fan and Zhang (2003); Manson (1972); Srinivas et al. (2007) Kuwarhara (1988); Ho (2008). Diaz et al. (2000)

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140	Bulb mite, *Rhizoglyphus echinopus* (Fumouze and Robin, 1868)	India, Australia, New Zealand, Russia	Leaf, bulb	Nymph and adult	Sandhu (1976); Hai fan and Zhang (2003)
141	Bulb mite, *Rhizoglyphus setosus* (Manson, 1972)	Taiwan	Leaf, bulb	Nymph and adult	Diaz et al. (2000); Chen et al., (2002)
142	Bulb mite, *Caloglyphus krameri* (Berlese, 1923)	Iran	Bulb	Nymphs and adult	Ostovan and Kamali (1995);
143	Storage mite, *Tyrophagus* sp	Pakistan	Bulb	Nymphs and adult	Sarwar (2012)
	Trombidiforms				
	Tetranychidae				
144	Red spider mite, *Tetranychus cinnabarinus* (Boisduval, 1867)	India	Leaf	Nymph and adult	Srinivas and Lawande (2004);
145	Two spotted spider mite, *Tetranychus cucurbitacearum* Sayed, 1946	Egypt	Leaf, bulb	Nymph and adult	Abo El-ghar and Osman (2009);
146	Two spotted spider mite, *Tetranychus urticae* C.L.Koch, 1836	Argentina	Leaf	Nymph and adult	Greco et al. (2006)
147	Brown wheat mite, *Petrobia latens* (Muller, 1776)	Egypt	Leaf, pseudo-stem and seed stalks	Nymph and adult	Abo El-ghar and Osman (1973)
	Eriophyidae				
148	Wheat curl mite, *Aceria tulipae* (Keifer, 1938)	Netherland, UK, China, Japan, United State, France, India	Leaf, bulb	Nymph and adult	Kiedrowicz et al. (2017); Navia et al. (2010); Conijin et al. (1996); Srinivas et al. (2007); Debnath and Karmakar (2013);
149	Wheat curl mite, *Aceria tosichella* Keifer, 1969	Russia, Brazil		Nymph and adult	Navia et al. (2006); Skoracka et al. (2013)

worm, *S. exigua*, the green looper, *Chrysodeixis acuta* Walker, *Liriomyza huidobrensis* (Blanchard), *L. sativa* Blanchard damaging onion green foliage and *Lasioderma serricorne* (F.) onion seeds are also known (Karuppaiah et al., 2019; Soumia et al., 2020; Verma et al., 2011).

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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BROAD SPECTRUM ACTIVITY OF ESSENTIAL OILS IN MANAGING STORED GRAIN PESTS

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ABSTRACT

The essential oils have broad-spectrum use over the world as non-chemical alternatives over the chemical fumigation in managing different stored grain insect pests. Exceptions are for organic products where use of fumigants is not possible then application of essential oils is the best preferred choice. However, their general commercial use needs to be explored over the world. The perusal of literature on essential oils indicated that the use of most effective natural products needs to be fully exploited for their potential use in the context of pest management in household and large scale grain storage.

Key words: Essential oils, broad-spectrum use, management, stored grain insect pests, management

Global population may increase to approximately 9.8 billion in 2050 than the existing 7.6 billion (Singh et al., 2021). The world may face a huge scarcity of food if the total world food production is not increased by 70% by the year 2050 (Anonymous 2017). Food grains and pulses constitute most consumed and the most common stored food products in the world, especially in the tropical and sub-tropical regions, hence occupying a crucial position in the resolution of food insecurity problems. Unfortunately, more than 70% of the produced grains are stored in villages in traditional structures such as earthen pots, silos, gunny bags, steel drums, and baskets (Mobolade et al., 2019). This often leads to loss of food grains and pulses, particularly in less developed countries where the need is the greatest (Kesavan and Swaminathan, 2008). There are various factors which cause postharvest losses of food grains and pulses, and damage inflicted by insects represents the highest threat. It has been reported that there is a wide range of losses of approximately 5-30% of the world's total agricultural production due to insect infestation alone on stored food grains (Prusky, 2011; Rajashekar et al., 2012). This wide variation in the estimation is partly due to different geographical zones, different climatic conditions, also the disparity in the major crops grown. There is also no concrete method to accurately estimate the losses (Singh et al., 2021). About 10% of post-harvest losses are incurred in food grains due to insects, rodents, micro-organisms, improper storage etc. In India, about 14 million tonnes (MT) of food grains worth Rs. 7000 crores have been lost annually. Among these, insects alone are responsible for losses of about Rs. 1300 crores (Banga et al., 2020).

The post-harvest losses due to storage and insect account for 2.0-4.2% (Kumar and Kalta, 2017). Insects not cause the losses in economic terms by consumption alone but also by spreading contamination. About 600 species of insects are occurred in stored grains and among these, about 100 species caused economic losses in stored grains (Patel et al., 1993). Several types of insects occurs which cause the infestation in stored food grains- primary, secondary and tertiary ones. Some insects behave as free-living, visualized to the eye during the first examination and hidden insects are those insects which exist in individual grains during their immature stage or due to their development in the grains. Insect's response to various natural and simulated features, classified into behavioral and metabolic responses. Movement of insects in stored grains in storage structures lies on environmental conditions. In the summer season, insect infestation occurs on the grain surface and disseminated in clumps throughout the mass, while in the winter season, insects flock in the lower and center portions of the storage structure. Attraction towards supportive conditions and evasion from fumigants, or treated surfaces comes into behavioral aspects whereas metabolic response includes the responses, which are used to promote or demote the development of insects (Bell, 2014). Primary insects are those which have the ability to bite or pierce intact and sound grains e.g. grain weevils (Curculionidae). They have the ability to break down the hard seed coat

of the whole grain and laid the eggs inside the kernel, and the growing larvae cause the infestation inside the kernel. Primary insects often develop and reproduce very quickly in the optimal conditions, which allows for large populations. They are usually more destructive than secondary pests, especially in short-term storage of food grains (Banga et al., 2018). Secondary insects follow the primary insects. They feed the grains that are broken by the primary insects, processed into products viz. flour, dal etc. or damaged by poor threshing, drying and handling e.g. red flour beetle, *Tribolium castaneum* (Herbst), one of the common secondary pests that eat wheat grains from the outside first. The presence of secondary pests often indicates that the grain is not in superlative condition and that measures should be implemented to protect the grain from a further decline in quality. Tertiary insects feed on broken grains, grain dust, and powder left by the primary and secondary insects. Confused flour beetle is a tertiary insect of whole grains. Also, it is a secondary insect of milled grains such as flour (Banga et al., 2019).

Singh et al. (2016a) evaluated different plant powers against rice weevil, *Sitophilus oryzae* (L.) on stored wheat and revealed neem and dharek kernel powders as the best. Chahal et al. (2016) studied the chemistry and insecticidal activity of bay leaf oil, its fractions and isolated compounds against red rust flour beetle, *Tribolium castaneum* (Herbst.) and revealed the adults of *T. castaneum* as more susceptible to eugenol. Their results also indicated that bay leaf essential oil may have potential to control *T. castaneum*. Singh et al. (2016b) evaluated the efficacy of three plant oils and revealed neem and eucalyptus oils as the most effective against lesser grain borer, *Rhyzopertha dominica* (L.) in stored wheat. Singla et al. (2018) used ajwain seed (*Trachyspermum ammi*) oil and some other derivatives as stored grain protectant against *S. oryzae*. Mohan et al. (2020) at TNAU developed some eco-friendly methods for stored grain/seed insect management.

Essential oils (EOs)

Widespread use of chemical insecticides in agriculture causing ecotoxicological, environmental and social consequences have led researchers to find out some more reliable and environmental friendly alternatives over the synthetic chemicals. In this context, the use of botanical extracts is attracting considerable interest both among the researchers and consumers. Among botanical extracts used as insecticides, EOs prove most promising alternative because of their worldwide availability and relative cost-effectiveness (Compolo *et al.* 2018). EOs are secondary metabolites synthesized by plants, and they play very important roles in plant defense (both against biotic and abiotic stresses) and signaling processes, including the attraction of pollinators and beneficial insects (Pavela 2015; Zurate & Salgueiro 2015). EOs are synthesized by plants both internally (secretory glands allocated inside the plants) as well as externally (secretory glands placed on the plant surface) (Svoboda & Greenaway 2003). They are produced by different plant organs such as trademark owners, herbs, buds, leaves, fruits, twigs, barks, seeds, woods, rhizomes, roots and can be accumulated in specific histological structures (glandular trichomes, secretory cavities, and resin ducts) (Asbahani *et al.* 2015; Fahn 2000). Plant species that produce EOs are called aromatic plants and are distributed worldwide; these plants (over 17,000 species) belong to a limited number of families: Asteraceae, Cupressaceae, Lamiaceae, Lauraceae, Rutaceae, Myrtaceae, Piperaceae, and Poaceae (Bruneton 1999). EOs are mainly constituted by monoterpenes and sesquiterpenes synthesized in the cytoplasm and plastids. All terpenes are synthetized via either the methylerythritol 4-phosphate (MEP) pathway or the mevalonate-dependent (MVA) pathway. Two (C5) isoprene precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), are involved in the terpene synthesis and the isoprene units determine their class (monoterpenes, C10; sesquiterpenes, C15) (Zebec *et al.* 2016). Sesquiterpenes contain 15 carbon atoms, and they are less volatile and have a higher boiling point than monoterpenes. As a consequence, fewer of them contribute to the fragrance of EOs (Husnu & Buchbauer 2015).

The EOs are constituted by a blend of 20 to 70 organic compounds, some of which represent more than 80% of the constituents as append, e.g., in Sweet Orange EO, the main compound, limonene reaches 88- 97% of the whole oil (Campolo *et al.* 2014). Generally, the main components characterize the biological activity of the EOs. The EOs are hydrophobic and generally lipophilic, and their density is often lower than that of water and they are soluble in organic solvents. Despite the numerous extraction methods used to obtain EOs from natural raw plant material, only four methods, such as (i) hydro distillation, (ii) steam distillation, (iii) dry distillation, and (iv) mechanical processes are considered in the European Pharmacopoeia and the International Standard Organization on EOs (Zurate and Slguerio 2015). The EOs can undergo physical treatments, which do not result in any significant change in its composition (e.g., filtration, decantation, and centrifugation) but

the resulted products consist of a blend of volatile compounds with a strong odor (Bakkali et al., 2008).

Extraction methods

Hydro distillation: It is one of the recent the standard EO extraction methods from plant materials like wood or flower, which is often used to isolate non water-soluble natural products with high boiling point. It involves the complete immersion of plant materials in water, followed by boiling. This method protects the oils extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. The advantage of this technique is that the required material can be distilled at a temperature below 100 °C. Okoh et al. (2010) studied the different extraction processes on yield and properties of EO from rosemary (*Rosmarinus officinalis* L.) by HD and solvent-free microwave extraction (SFME). The total yields of the volatile fractions obtained through HD and SFME were 0.31% and 0.39%, respectively. HD oil contained more monoterpene hydrocarbons (32.95%) than SFME-extracted oil (25.77%), while higher amounts of oxygenated monoterpenes (28.6%) were present in the oil extracted by SFME in comparison with HD (26.98%). Golmakani and Rezaei (2008) studied the microwave-assisted HD (MAHD), which is an advanced HD technique utilizing a microwave oven in the extraction process. MAHD was superior in terms of saving energy and extraction time 75 min, compared to 4 h in HD). Ohmic-assisted HD (OAHD) is another advanced HD technique (Gavahian *et al.* 2012). OAHD method had the extraction time of 24.75 min, while HD took 1 hour for extraction of EO from thyme. No changes in the compounds of the EOs obtained by OAHD were found in comparison with HD. Hydro distillation method is considered the simplest one to obtain EOs from the plant material by immersion of biomass in boiling water. The oil contained in the oil cells diffuses by means of osmosis in the hot water; then the steam, produced by boiling water, carries the oil vapors in a condenser. The condensed EOs are separated from water by decantation.

Steam distillation: In this, the vapor is supplied in such a way that liquid water does not come into contact with the vegetable raw material. In the simplest version, steam is generated by water added in the lower part of the distiller; the plant raw material is separated from the liquid water by a perforated grid. The steam that passes through the plant material carries the oil vapors, and after passing through a condenser, EO is separated from water by decantation. It is the most widely used method for plant EO extraction (Reverchon and Senatore, 1992). The proportion of EOs extracted by steam distillation is 93% and the remaining 7% can be further extracted by other methods (Masango, 2005). The heat applied is the main cause of burst and break down of cell structure of plant material. As a consequence, the aromatic compounds or essential oils from plant material are released (Perineau et al., 1992; Babu and Kaul, 2005). The temperature of heating must be enough to break down the plant material and release aromatic compound or essential oil. A new process design and operation for steam distillation of EOs to increase oil yield and reduce the loss of polar compounds in wastewater was developed by Masango (2005). The system consists of a packed bed of the plant materials which sits above the steam source. Only the steam passes through it and the boiling water is not mixed with plant material. Thus, the process requires the minimum amount of steam in the process and the amount of water in the distillate is reduced. Also, water soluble compounds are dissolved into the aqueous fraction of the condensate at a lower extent (Masango, 2005). Yildirim et al. (2004) reported that the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activities of EOs from steam distillation process were markedly higher than those of oils extracted using hydro distillation (HD).

Solvent extraction: This method employs food grade solvents like hexane and ethanol to isolate EOs from plant material. It is best suited for plant materials that yield low amounts of essential oil, that are largely resinous, or that are delicate aromatics unable to withstand the pressure and distress of steam distillation. This method also produces a finer fragrance than any type of distillation method. Through this process, the non-volatile plant material such as waxes and pigments, are also extracted and sometimes removed through other processes. Once the plant material has been treated with the solvent, it produces a waxy aromatic compound called a "concrete." When this concrete substance is mixed with alcohol, the oil particles are released. The aforementioned chemicals used in the process then remain in the oil and the oil is used in perfumes by the perfume industry or for aromatherapy purposes.

Conventional solvent extraction has been implemented for fragile or delicate flower materials, which are not tolerant to the heat of steam distillation. Different solvents including acetone, hexane, petroleum ether, methanol, or ethanol can be used for extraction (Areias et al., 2000; Pizzale et al., 2002; Kosar et al., 2005). For general practice, the solvent is mixed with

the plant material and then heated to extract the essential oil, followed by filtration. Subsequently, the filtrate is concentrated by solvent evaporation. The concentrate is resin (resinoid), or concrete (a combination of wax, fragrance, and essential oil). From the concentrate, it is then mixed with pure alcohol to extract the oil and distilled at low temperatures. The alcohol absorbs the fragrance and when the alcohol is evaporated, the aromatic absolute oil is remained. However, this method is a relatively time-consuming process, thus making the oils more expensive than other methods (Li et al., 2009). Essential oil with antioxidant activity from *Ptychotis verticillata* was extracted using solvent extraction method by El Ouariachi et al. (2011). The oil was dominated by phenolic compounds (48.0%) with carvacrol (44.6%) and thymol (3.4%) as the main compounds. Ozen et al. (2011) studied the chemical composition and antioxidant activity of separated EOs from *Thymus praecox* subsp. *skorpilii* var. *skorpilii* (TPS) extracted using different solvents. TPS essential oil was found to contain thymol (40.31%) and o-cymene (13.66%) as the major components. The ethanol, methanol, and water extracts exerted significant free-radical scavenging activity. The water extract has the highest total phenolics (6.211 mg gallic acid/g dry weight) and flavonoids (0.809 mg quercetin/g dry weight). Moreover, Sarikurkcu et al. (2009) reported that the water extract exhibited higher antioxidant activity than other extracts (hexane, dichloromethane, ethyl acetate, and methanol). However, solvent residue could be retained in the final produc due to incomplete removal. This may cause allergies, toxicity, and affect the immune system (Ferhat et al., 2007).

Cold-press extraction: This method is also called expression or scarification and is used for citrus peels in particular. In this the whole fruit is placed in a device that mechanically pierces it to rupture the essential oil sacs, which are located on the underside of the rind. The whole fruit is pressed to squeeze out the juice and the oil. The oil and juice that are produced still contain solids from the fruits, such as the peel, and must be centrifuged to filter the solids from the liquids. The oil separates from the juice layer and is siphoned off into another receptacle. The essential oil and pigments run down into the device's collection area (Benjakul and Tongnuanchan, 2014).

Plant volatiles as alternative

Plants being one of nature's chemical producers provide several bioactive organic chemicals whose major role in plants was found to be defensive, especially against insect pests (Isman, 2006). Those organic bioactive compounds provide an odor that is typically volatile in nature and hence termed as PVOC (plant volatile organic compounds). However, considering the dangers associated along with the chemical insecticides, use of plant components with no or fewer threats forms the most viable option for the effective control measures against the various pests of tropical agricultural systems (Rajashekar et al., 2016). There are various plant species which embody a rich source of phytochemicals that could be explored for use as insecticides (Green et al., 2015). The secondary metabolites of plants were once considered to be waste products. However, the important role played by them in the plant defense mechanisms make them as one of the most interesting aspects of crop protection (Bennett and Wallsgrove, 1994). In addition, they also act as attractant or repellent in insect-pest management. Secondary metabolites are known to influence the growth and development, ecdysis, mating behavior, fertility, and adult emergence of insect pests. Numerous defensive chemicals of plant origin have already been identified. Plant volatiles organic compounds are generally much safer to human beings and to the environment. There is also less chance of developing resistance to the different botanical pesticide by the insect pests since these botanical pesticides possess a mixture of chemical components of a complex nature. Hence, plant-derived molecules could thrive in the fight against insecticide-resistant insect pests. It is well documented that farmers, especially in the developing countries like Asia, Africa, and other parts of the world are using botanicals as a measure to protect stored grains from bruchid infestation with varying degrees of success (Rajashekar et al., 2010) The secondary metabolites are often involved in plant defense mechanism, but not directly involved in growth, development, or reproduction of plant. Secondary metabolites include terpenoids, phenolics and alkaloids and these phytochemicals possess enormous potential to act as biopesticides. Over 2000 plant species boast pesticidal properties against several stored grain pests. Several authors also set forth various plant products along with their efficacy against the stored grain pests. These plant-derived botanicals are generally used in the form of aqueous/solvent extracts, powders, slurries, volatiles and oils, or as shredded segments. Hence, plant-derived botanicals hold promise as an alternative to synthetic insecticides to lessen the negative impact of the pesticide on the environment.

Chemistry of EOs

The EOs are naturally found in plants as secondary

metabolites which play an important role in plant defense system against microorganisms, insects, herbivores and allelopathic interactions (Bakkali et al., 2008). Previous studies have reported that volatile compounds of EOs can be classified into four groups: (a) Terpenes, (b) Benzene derivatives, (c) Hydrocarbons and (d) Other miscellaneous compounds. Based on the number of isoprene units in the chemical structures of terpenes, EOs are classified into (a) Hemiterpenes (1 unit, C5), (b) Monoterpenes (2 units, C10), (c) Sesquiterpenes (3 units, C15), (d) Diterpenes (4 units, C20) and consequently, it has been found that most terpenes in EOs are monoterpenes (C10H16) and sesquiterpenes (C15H24). However, monoterpenoids are the most terpenes and represented by 90% of EOs (Zuzarte and Salgueiro, 2015). Monoterpenes have a chemical structure which varies greatly along with various functions. Monoterpenes and their related chemical structure compounds have 10-carbone hydrocarbon. These chemical structures divided into (1) Acyclic alcohols such as linalool, geraniol and citronellol, (2) Cyclic alcohols such as menthol, isopulegol and terpeniol, (3) Bicyclic alcohols such as borneol and verbenol, (4) Phenolic compounds such as thymol, carvacrol, (5) Ketones such as carvone, menthone and thujone, (6) Aldehydes such as citronellal, citral, (7) Acids such as chrysanthemic acid and (8) Oxides such as cineole (Tripathi et al., 2009).

Biopesticides

For two decades, EOs are used as insecticides to control insects, however, they have not reached their full potential because they are highly volatile and bearing low residual activity (Orlando et al., 2018). They are considered safe, eco-friendly and compatible with biological control programs and have low mammalian toxicity. Also, EOs available worldwide have their low to moderate cost. The main active constituents with insecticidal activity are monoterpenes, sesquiterpenes and related phenyl propenes. The EOs can be applied as pesticides as they are or as their active components "Active ingredient" or as co-adjutants in pesticide formulations. However, they can be acted as a contact insecticide, causing changes in the pathways of biochemical metabolism of the insect, knockdown and rapid death (Saxena et al., 1992), fumigants (Shaaya et al., 1997), repellents and antifeedant.

Toxicity and mode of action

The plant products with potent insecticidal activity could be used for the control of stored beetles. Many authors have reported that plant volatiles are toxic and effective in enhancing the mortality of adult beetles infesting stored grains. The plants are known to produce diverse secondary phenolic compounds which could have played a major role in inflicting the contact toxicity and mortality effect to these stored grain insects (Lattanzio et al., 2006). Kim et al. (2003) reported that the extract from the Chinese cinnamon, *Cinnamomum cassia* (L.); Lauraceae bark and oil; scurvy grass, *Cocholeria auracaria* (L.); mustard oil, *Brassica juncea* (L.) showed potent insecticidal activity against pests like *C. chinensis*. The fumigation is one of the methods in stored products pest management and it is done for killing the insects or to avoid further damage to the infested commodities. Fumigants are substances that vaporize at the temperature above 5^0C. They act in gaseous or smoke form with high penetration power and usually applied in an enclosed/airtight system. In the vapor phase, plant volatile organic compounds can penetrate through the insect's respiratory system and exhibit its toxic effect (Choi et al., 2003). The fumigant activity of plant products may be due to different functional groups attached to these volatile chemicals which enable them to persist in a closed environment for a longer time (Zang et al., 2011). One of the advantages of bio-fumigants is that it has the potential to provide a novel mode of action against insects that could nullify the danger of cross-resistance as well as offering a new lead for the design of target-specific molecules. PVOC from many plant extracts and essential oil consists of alkanes, alcohols, aldehydes, terpenes and terpenoids especially monoterpenoids. Phytochemicals which exhibit effect on insects in terms of their toxicity, repellency, antifeedant, fumigant, growth inhibitors, suppression of reproduction and reduction of fecundity.

Phytochemicals are known to affect growth, development, and metamorphosis of insect. These cause irreversible changes in the physiology and behavior of insect, such as reduction in weight of larvae, pupae and adult, as well as prolonged larval and pupal periods (Rosenthal et al., 1995). Several plant volatiles with potential insect growth regulator and sterilant characteristics have been assessed in this respect. Various plant extracts showed inhibition in percentage pupation upon larval treatment. The EOs from *Cymbopogon schoenanthus* (Spreng) (Poaceae) show inhibition of growth and development in all life stages of *C. maculatus*. The Plant-derived botanicals were also found to inhibit the development of eggs and immature stages inside grain kernels. Aqueous extracts of common cocklebur *(Xanthium strumarium*

(L.) (Asteraceae) leaf was also reported to show several insecticidal properties including toxicity, repellency, inhibition of fecundity and adult emergence of the insects and grain protection against *C. chinensis* (Roy et al., 2014).

Several investigations have been carried out to screen various plant extracts for their juvenilizing effect. Rani and Jamil (1989) discovered that plant extracts of water hyacinth containing a juvenile hormone analogue that causes abnormal moulting and metamorphosis of stored grain insects. Their role in regulating the reproduction of insect is also well studied. Lingampally et al. (2012) reported Solasodine @ 1 µg/µl to inhibit moulting and induce several morphogenetic abnormalities leading to the death of 5th instar larvae of *Tribolium confusum* (Jacquelin du Val) (Tenebrionidae) during moulting and could affect adult emergence. Oviposition deterrents are chemicals which prevent or avoid insect from egg laying. Oviposition deterrents have huge potential to prevent insect infestation and can offer the first line of defense against insect pests. Plant volatiles are generally used as cues by ovipositing females for locating host plant or substrates. Many experts have reported the involvement of PVOC in part or completely preventing egg laying as well as the emergence from the laid eggs on stored grains by different families of insect pests. The chemical compound, 1, 8 cineole isolated from EOs and their volatile component of various plants within Lamiaceae family was found to affect the oviposition rate of various insects (Koschier and Sedy, 2001). Used of garlic oil as an oviposition deterrent is also common (Yang et al., 2012). EOs of *Eucalyptus citriodora* (Hook.), *E. globulus* (Labill.) and *E. staigeriana* (F. Muell.) showed severe effects on the oviposition thereby reducing the viability of eggs and insect's emergence of *Zabrotes subfasciatus* (Chrysomelidae) and *C. maculatus* (Tapondjou et al., 2005). The EOs of other plants, viz., *Trachyspermum ammi* (L.), *Anethum graveolens* (L.) and *Nigella sativa* (L.) have affected the oviposition potential and delayed the developmental period of *T. castaneum*. It also caused deformities in insect metamorphosis. *Laurus nobilis* (L.) (Lauraceae) and *Rosmarinus officinale* (L.) (Lamiaceae) are known to cause egg mortality (Isikber et al., 2006). In a study, the effect of finely powdered and intact dried leaves of *Ocimum canum* Sims (Lamiaceae) on adults of *Zabrotes subfasciatus* (Roheman) in dried Pinto beans were determined. It was reported that the finely powdered dried leaves completely suppressed the oviposition at 2% W/W, with an EC50, of 0.45% W/W and also there was no effect of intact dried leaves reported on the insect's population. In a similar report, powdered and intact dried leaves from four locally grown plant species, i.e., *Chenopodium ambrosioides* (L.) (Amaranthaceae), *Tagetes minuta* L. (Asteraceae), *Azadirachta indica* A. Juss (Meliaceae), *C. lusitanica*, applied at a rate of 1.5 kg per 100 kg beans (*Phaseolus vulgaris*) against *A. obtectus* and *Z. subfasciatus* was compared under laboratory conditions. *C. ambrosioides* was found to be the most effective with 100% mortality of adult insects in less than three days and no progeny. *T. minuta* applied as powder also increased mortality and reduced oviposition and progeny production significantly. While *A. indica* or *C. lusitanica* applied as powder or as whole leaves showed no significant effects upon mortalities, oviposition rate, or progeny production compared with control treatments (Paul et al., 2009).

The feeding deterrents are the chemical substances that disrupt the feeding behavior of insect by making the treated food unappealing or unpalatable. Antifeedants can also induce cessation of feeding either temporarily or permanently. The presence of certain chemicals in plants prevents insects from feeding on them, thus leads to starvation of the insects and, eventual mortality. A very distinctive feature of antifeedants is the way it inflicts effect on insects when in contact with such substances. Antifeedant attempts to eliminate insects without ever disturbing the ecological balance. Antifeedant don't kill the target insect, rather it allows them to be available for their natural enemies. The deleterious effects could be activated to serve a novel role in the management of stored grain insect pest. The identification of azadirachtin and neem seed extracts, during 1970s and 1980s, as potent feeding deterrent pave the way for natural antifeedant (Casida 2012). The EOs from *Gaultheria procumbens* (L.) (Ericaceae) show potent antifeedant properties against Coleopteran insects S. *oryzae* and *R. dominica* (Kiran & Prakash 2015). The feeding behavior of three stored grain insects, *S. oryzae, R. dominica* and *T. castaneum* were reported to change significantly when flavanoids compounds, Isoglabratephrin, (þ)-glabratephrin, tephroapollin-F and lanceolatin-A isolated from *Tephrosia apollinea* (Delile), were treated at different test concentrations of 0.65, 1.3 and 2.6 mg g^{-1}. The relative growth rate and efficiency of conversion of ingested food were also significantly reduced by all the treated insects.

These are the chemical substances that protect plants or stored grain products from insect's damage by rendering the stored grains as unattractive, unpalatable

or offensive to the infesting insect pests. PVOC mostly affect adult beetles causing them to flee from treated grains, or not to invade it at all. The properties of repellency of plant volatiles could have an important implication in the traditional postharvest storage system. Generally, they are available locally and it makes them further an attractive candidate in the management of stored-grain insect pests. The α-terpineol, pulegol and germacrol volatile compounds isolated from *Baccharis salicifolia* Ruiz & Pav. (Asteraceae) showed potent repellent properties against *T. castaneum*. The ar-turmerone isolated from *Curcuma longa* (L.) (Zingiberaceae) rhizomes were reported to show effective repellent activity against *S. zeamais*. Different solvent extracts (Hexane, chloroform, ethyl acetate) of three widely grown plants like *Sphaeranthus indicus* (L.) (Asteraceae), *Tephrosia purpurea* L. (Fabaceae) and *Prosopis juliflora* (Sw.) DC. (Fabaceae) were reported to show repellent activity against *T. castaneum*. Repellent activity of hexane extract of *P. juliflora* were reported with EPI value in 2.5% 0.11 and 0.33 at 1 hours and 6 hours, respectively, while the EPI value of chloroform extract of *T. purpurea* 2.5% at 6 h was 0.17. For *S. indicus* the EPI value was 0.65 at 2.5% and 6 hours (Pugazhvendan et al., 2012). (Z)-asarone is an active constituent isolated from the ethanolic extract of *Acorus calamus* (L.) (Acoraceae). This compound is reported to have strong repellency against *S. zeamais* (Yao et al., 2008). The repellent property of EOs of *Callistemon lanceolatus* (Sm.) Sweet (Myrtaceae) and *Lippia alba* (Mill.) (Verbenaceae) against *C. chinensis* is also well documented. The EOs of aromatic plants such as *Cymbopogon fexuosus* (Paoceae), *C. winterianus* (Paoceae) and *C. martini* (Paoceae) @ 1.41 µL/ cm^2 were reported to have strong repellent effect against *T. castaneum.*

Substance or agent that has the potential to kill eggs especially the eggs of insects, mites etc. are considered to have ovicidal effect. Many plants are known to possess the ovicidal property. This property of plants is of great importance in the management of insect-pests at every stages of their life cycle, thereby preventing the damage caused by other stages (Hong et al., 2018). Application of botanicals on eggs could tremendously reduce the number of adult emergence which is probably due to either chemical toxicity and/ or physical properties, which cause changes in surface tension within the egg similar to those of oils. Salunke et al. (2005) revealed that flavonoids which were isolated from *Calotropis procera* (Ait.) were toxic to the eggs of *C. chinensis* with 100% progeny suppression at 10 mg/mL concentration. The EOs of Indian dill, *Anethum sowa* (L.) (Apiaceae) were also reported to show ovicidal effect on eggs of C. maculates. L-menthol is an active component, isolated from the essential oil of *Mentha arvensi* L. (Lamiaceae), which has high ovicidal effect on eggs of *T. castaneum* (Aggarwal et al., 2001). The EOs of cardamom (*Elletaria cardamomum*) L. (Zingiberaceae), *Cinnamomum zeylanicum* Blume (Lauraceae), *Sygium aromaticum* L. Merrill. et. Perry) (Myrtaceae), *Eucalyptus* spp. and *Azadirectica indica* A. juss (Meliaceae) were also found to have ovicidal effects on eggs of *T. castaneum.*

Chemosterilants are the substances which deprive insects of their ability to reproduce. Such substances produce irreversible sterility without affecting their mating behavior or its life span but only prevent the production of F1 progeny. In some cases, eggs may not be laid, fail to hatch, larvae may not pupate, or the pupal development will be incomplete. Chemosterilants cause damage to the ovaries resulting in inhibition of egg formation (Beroza and LaBrecque, 1967) Thus, ovarian development is prevented, and it leads to sterility. Chemosterilants has proven to be an important aspect of integrated pest management programs as it reduces the occurrence of pest-resistant. Saxena et al. (1977) found that asarone isolated from the rhizomes of *A. calamus* possessed insect chemosterilant properties, causing inhibition of insect's interstitial cell activity. The active principle, 1, 3, 7-trimethylxanthine isolate from seed extract proved effective as chemosterilant for *C. chinensis.*

Like other toxic compounds, plant derived insecticides may also induce changes in the behaviour of insects. They either stimulate or reduce the insect's mobility or flight patterns, and even cause physiological alterations. Naturally, insects will tend to move away from the insecticide exposed area as soon as the presence of toxic compounds is detected. Besides EOs are known to inhibit acetylcholine esterases enzyme on insect's nervous system while also disrupting the GABAergic (Bloomquist et al., 2008) and aminergic transmissions (Kostyukovsky et al., 2002). However, EOs when given in sub lethal dose on insects can contribute to the development of resistance against traditional insecticides that could also compromise the management strategy of insect-pests. In an experiment, Haddi et al. (2015) investigated the toxicity and locomotory as well as respiration responses induced by EOs of clove and cinnamon on *S. zeamais*. It was reported that *S. zeamais* populations gave stimulatory

response in median survival time when exposed to sublethal dose. The respiratory rates of *S. zeamais* (i.e., CO_2 production) were significantly reduced under low concentrations of the EOs.

The mechanism of toxicity, site of action of EOs and their chemical compounds as bio-insecticides were investigated (Bakkali et al., 2008). They have insecticidal, repellent, antifeedant and IGR activities. This fact shows that the EOs can disrupt insect physiology in different ways. However, the activity of EOs as insecticides caused the inhibition of acetylcholinesterase (AChE) or by blocking the octopamine receptors (Enan, 2001). Also, γ-aminobutyric acid (GABA) receptor was suggested as one of the mechanisms of EOs induced toxicity in insects. They can interfere with GABA-gated chloride channels in insect. For example, some monoterpenes such as thujone can induce the neurotoxic effects by acting on GABA receptors in insects (Ratra & Casida 2001). Besides, these modes of action of EOs on insects, some effects on the hormone and pheromone system and cytochrome P450 mono oxygenase were reported. Infact, octopamine is a neurotransmitter, neurohormone and circulating neurohormone-neuromodulator. Its disruption affects working of the nervous system in insects. The octopamine receptor was not found in vertebrates. Therefore, EOs as bioinsecticides has selective toxicity to mammalian. Concerning the anti-cholinesterase effect of EOs, the mechanism of neurotoxic action and symptoms are similar to that induced by organophosphates and carbamate insecticides (Isman, 2000). The symptoms shown are hyperactivity, convulsions, tremors and then paralysis (Enan, 2001). Several studies point out that EOs and monoterpenoids induce mortality by inhibiting AChE activity in insects. The neurotoxic effect of EOs may be due to the inhibition of AChE at the hydrophobic site168. However, some monoterpenoids are anticholinesterasic e.g., pulegone, 1, 8-cineole169, fenchone, carvone and linalool (Lopez *et al* 2010). In addition, the toxicity of EOs constitutions has resulted in the octopaminergic nervous system of insects. In contrast, Emekci et al. (2004) reported that monoterpenes can have effect on many targets in insects with numerous mechanism of toxicity.

Nanoinsecticides: Although, the promising activities of EOs against many insects, some problems were also registered, e.g., EOs volatility, water solubility and oxidation, that playing an important role in the EOs activity, application and persistence. Therefore, these problems must be resolved before using the EOs as an alternative to synthetic pesticides for pest control (Marrio et al., 2002). New formulations with nanotechnology "Nano-formulation" can resolve these problems. The new trends for using nano formulation lead to protect the Eos' from degradation, increase their residue half-life by reducing the evaporation. They can achieve a controlled release of EOs and ease of application and handling. These nano-formulations can enhance EOs activity due to the small particle size. It had a high surface area, solubility and mobility. Due to their solvent elimination, they have low toxicity to mammals (Kah et al., 2013). However, polymeric nanoparticles are the most promising for EOs nano-formulations. Anjali et al. (2010) reported that the insecticidal activity of neem oil was increased in nano-emulision formulation. This effect can be due to the smallest droplet size of EO nano-emulision (31.03 nm). Yang et al. (2009) showed that loaded nanoparticles with garlic EOs are active to control *T. castaneum*. New nanotechnology methods were planned to control *H. armigera*. As a result of this new method EO *of Artemisia arborescens* was stable. This stability can be due to formulation of EO with solid lipid nanoparticles and developed an emulsion. This emulsion has got better stability and insecticidal activity. The nano-encapsulation shown high repellent activity than EOs, e.g. nano-encapsulation of *Artemisia* oil and show more activity than normal oils at concentration of 1.9 ppm. The activity of nano-capsule was 80, whereas 62% for pure oil against *P. xylostella* (Negahban et al., 2014). Ebrahimifar et al. (2020) evaluated the effects of essential oil from *Ferulago angulata* (Schlecht.) The essential oil from *F. angulata* could be used for reducing *T. castaneum* and *R. dominica* populations without adverse effects on seed viability. Giunti et al. (2021) studied bioactivity of essential oil-based nano-biopesticides towards *R. dominica*. The results demonstrated that habituation could occur for repellent EO-based formulations, thus this behavioral process can reduce the effectiveness of these kind of treatments against *R. dominica* and should be considered to articulate adequate IPM programs against stored product pests.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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FUNGAL ENDOPHYTE-PLANT-INSECT INTERACTION: A TRIPARTITE ASSOCIATION NEEDING ATTENTION

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ABSTRACT

Fungal endophytes are microfungi which infect the aerial and underground living tissues of plants and live within them without causing any disease symptoms. Their association with plants confers several benefits to plants and enhances their innate ability to tolerate abiotic and biotic stresses. Despite their universal occurrence, their ability to improve plant performance and capacity to produce numerous novel bioactive compounds, information on the ecology of fungal endophytes is incomplete. The interaction of these fungi with plants, plant-associated organisms, cooccurring endophytes (other fungi and bacteria), and the environment is little understood. This article highlights one such interaction among plants-fungal endophytes-plant-feeding insects that needs attention.

Key words: Entomopathogenic fungi, plant-feeding insects, plant microbiome, microfungi, plant performance, ecology, interactions

Fungi and insects are the most-speciose and most-widespread organisms. They not only interact among themselves but also with plants. Insect-fungus interactions cover the entire gamut of symbiosis from mutualism to antagonism. Different species of Hypocreales (Ascomycota), such as *Beauveria bassiana, Metarhizium robertsii*, and *Ophiocordyceps unilateralis*, are popular examples of pathogenic fungi that are associated with multiple taxa of arthropods and are presently exploited as biological-management agents to manage pestiferous-arthropod populations. When fungi and arthropods collectively interact with plants, such associations impact on the fitness of plants. Spores and mycelial fragments of plant-pathogenic fungi either sticking to insect bodies or ingested by insects are transported to healthy plant tissues (Willsey et al., 2017). An insect can also acquire a pathogenic fungus along with food, which then circulates within the insect body and reaches its mouthparts, and finally enters the plant when the insect feeds on the plant (Wielkopolan et al., 2021). Insect-fungus interactions can be categorized from 'symbiosis' (= mutualism) to 'antagonism'. The involvement of the plant as the third component in such associations, further confounds the interaction scene. Examples of such a tripartite interaction include the modification of flower nectar by different species of yeasts (*e.g.*, *Metschnikowia gruessii, M. reukaufii [Saccahromycetales: Metschnikowiaceae], Aureobasidium pullulans* (Dothideales: Dothioraceae), *Hanseniaspora uvarum* (Saccharomycetales: Saccaharomycetaceae), and *Sporobolomyces roseus* [Sporidiobolales: *Incertae Cedis*) to make it more attractive nutritionally and by scent, for the pollinating *Apis mellifera* (Hymenoptera: Apidae) and parasitic *Aphidius ervi* (Hymenoptera: Braconidae) (Sobhy et al., 2018).

Generally, infection by a fungus alters the host plant's metabolism leading to either enhanced levels of defence compounds or diminished levels of nutrients (Raman and Suryanarayanan, 2017). Fungal-plant pathogens modify the phenotypes, nutritional profiles, and the chemistry of volatile-organic compounds (VOCs) of their host plants as a tactic to attract arthropod vectors enabling their dispersal. For example, the plant-pathogenic *Fusarium verticillioides* (Hypocreales: Nectriaceae) modifies the chemistry of its host-plant's mechanism of biosynthesis of volatile compounds making them attractive for the caterpillars of *Diatraea saccharalis* (Lepidoptera: Crambidae). Furthermore, this fungus influences both the host plant and insect's lifecycle to promote its infection and spread (Franco et al., 2021). Similarly, colonization of roots by 'mycorrhizae', the soil-inhabiting fungi that usually bear a symbiotic relationship with the roots of diverse flowering plants. Such mycorrhizal associations distinctly alter multiple morphological, physiological, and biochemical characteristics of the host plant, such that the overall performance of those insects that feed on the plant

is influenced either positively or negatively, which largely depends on the feeding mode and diet range of the insect taxon and associated mycorrhizal species (Koricheva et al., 2009). Plant-insect interactions have been studied in great detail because the plant-feeding arthropods affect production of crop plants in particular by feeding on the aerial and below-ground plant parts (Sharma et al., 2017). Moreover, the role of arthropods as vectors of plant pathogenic microbes is well known (Agrios, 1997).

Presently, we know too well that flowering plants harbour different species of bacteria and fungi, which do not manifest any disease symptoms. Such nonpathogenic fungi and bacteria are termed the 'endophytic fungi' and 'endophytic bacteria', respectively. Species of *Colletotrichum* (Glomerellales: Glomerellaceae), *Phomopsis* (Diaporthales: Valsaceae), *Phyllosticta* (Botryosphaeriales: Botryosphaeriaceae), and *Xylaria* (Xylariales: Xylariaceae) are common endophytic fungi and *Pseudomonas* (Pseudomonadales: Pseudomonadaceae), *Burkholderia* (Burkholderiales: Burkholderiaceae), and *Bacillus* (Bacillales: Bacillaceae) are common endophytic bacteria, which colonize the leaves of almost all terrestrial plants. Few algae (Chlorophyta) such as *Cephaleuros* (Trentepohliales: Trentepohliaceae) also occur as endophytes in some plants. Endophytic lifestyle is so common among fungi such that plant-fungal endophyte relationship could be ancestral in nature. Ancestors of fungi possibly were endophytes such that they infected plants and switched their lifestyle to either saprobes or pathogens. This hypothesis is supported by fossil evidence and the lifestyle shifts, common among fungal endophytes (Bhunjun et al., 2023). It is estimated that 1-3 million fungal-endophyte species exist, although presently only c. 150,000 species have been determined and named (Bhunjun et al., 2023).

Based on the types of symbiosis they manifest, the fungal endophytes are presently classified into four types (Rodriguez et al., 2009): 1. Species of the Clavicipitaceae cause systemic intercellular infections in the shoots of some cool-season grasses and are transmitted vertically through seeds. Here, the endophyte fungal mycelium grows systemically within the host plant and enters its seeds; when such endophyte infected seeds germinate, the endophyte mycelium within the seed grows systemically within the seedling and continues to grow as the plant matures only to infect its seeds again. All the other three classes of endophytes are non-Clavicipitaceous fungi. The type 2 endophytes colonize the roots, stems, and leaves and are transmitted through seed coats and rhizomes. The type 3 endophytes infect the above-ground tissues of vascular and non-vascular plants. They are hyper-diverse, ubiquitous and their spores are transmitted to new plant hosts through air or insect vectors (horizontal transmission). The type 4 endophytes colonize the roots and have dark septa in their mycelium. In this opinion article, I will refer to the type 3 fungal endophytes to underscore the need to study the tripartite interaction between the plant, endophyte and insect.

Fungal endophyte-plant interaction

A leaf is usually and entirely colonized by 10-15 species of fungal endophytes. These could be either symbiotic or latent pathogens (Hyde et al., 2019). The ecological functions of fungal endophytes and their interaction with plants and simultaneously with other associated endo-microbes are not clear (Suryanarayanan, 2020). A few studies have shown that infection by a fungal endophyte protects the host plant from other pathogens (Arnold et al., 2003) by upregulating many defense genes of the host plant, thus making it more resistant to pathogens (Mejía et al., 2014). Furthermore, fungal endophytes are a novel source of bioactive secondary metabolites exhibiting interesting bioactivities including anticancer, anti-fungal and anti-diabetic, as proved by their ability to produce such molecules in aseptic cultures in the absence of their plant hosts (Schulz et al., 2002; Suryanarayanan et al., 2009; Aly et al., 2010). Apart from producing novel metabolites without the association with their host plants, a few fungal endophytes are capable of producing the secondary metabolites of their host plants; these include the anticancer metabolites such as paclitaxel (Stierle et al., 1993) and camptothecin (Kusari et al., 2009; Kusari and Spiteller, 2012).

Fungal endophytes also are a source of novel enzymes of industrial importance such as inhibitor-resistant biomass-degrading enzymes (Sengupta et al., 2017; Suryanarayanan et al., 2012) and anticancer enzymes (Nagarajan et al., 2014). A few recent studies reveal that fungal endophytes increase the fitness of their host plants by increasing their tolerance to abiotic stressors (Suryanarayanan and Uma Shaanker, 2021). For instance, a fungal endophyte isolated from the salt-tolerant *Oryza sativa* var. *Pokkali* (Poaceae) water-logged coastal parts of Alappuzha, Thrissur, and Ernakulam districts of Kerala, when inoculated in a salt-sensitive variety of *O. sativa* enables it to tolerate salt stress and to grow in salt water (Sampangi-Ramaiah et

al., 2020). Similarly, fungal endophytes from plants of geothermal habitats when transferred to heat-sensitive plants confers heat tolerance to them (Rodriguez et al., 2008).

Insect partners in the complex association

Despite being a consistent endosymbiontic component of plants, the association and interactions of plants-fungal endophytes-plant-feeding insects has received little attention. This could be due to that some patterns are apparent while considering interactions between plants and fungi; however, when the plant-feeding insects are considered in the equation, no discernible pattern is obvious probably due to the increased level of complexity involving three components (Raman and Suryanarayanan 2017). Considering the extensive width of taxonomic variability, the endophytic fungi and their ability to infect nearly all of the species of the Plantae, numerous patterns of interactions involving plants, fungi, and insects become evident (Suryanarayanan, 2013). For instance, it is highly likely that a direct relationship exists between insects and fungal endophytes on a flowering plant. One indirect evidence for this comes from the study of endophyte status of *Bt* (*Bacillus thuringiensis*) gene-incorporated varieties of *Gossypium hirsutum* (Malvaceae) (Suryanarayanan, 2013). When compared with the wild type, different tissues *Bt*-incorporated *G. hirsutum* had strikingly lesser density of fungal endophytes. This suggests that insects visiting *G. hirsutum*, for either pollinating or feeding possibly transmit the inoculum of the endophyte fungus (Suryanarayanan et al., 2011).

Moreover, injuries inflicted by insects on plants possibly act as portals for the fungal endophyte infection. Plant-feeding insects visiting and damaging plants are possibly influenced by the metabolites produced by the fungal endophyte–plant association. It is known that the fungal pathogen *F. verticillioides* enters the host plant through the injuries caused by the feeding activity of *Diatraea saccharalis* (Lepidoptera: Crambidae). Once established in the host plant, the fungus emits volatile compounds to attract the immatures; when the caterpillars become adults, the fungus is vertically transmitted through their offspring, which continues the cycle by inoculating the fungus into healthy plants (Franco et al., 2021). Extending this example, studies are needed to know if fungal endophytes similarly alter plant metabolism and physical properties to either attract or deter insect visits to host plants (Li et al., 2014). Supporting of certain species of fungal endophytes deters plant-feeding insects from attacking the host thus enhancing plant performance. For example, the foraging behaviour of *Atta colombica* (Hymenoptera: Formicidae) in Panama isthmus is influenced by the endophytes harboured in the leaves of *Merremia umbellata* (Convolvulaceae). The presence of endophytes negatively affected the foraging preferences of populations of *A. colombica*, although the reasons for avoidance of endophyte-rich host leaves by *A. colombica* are not clear as yet (Van Bael et al., 2009). These results suggest that interactions among fungi in the leaf endobiome and between the leaf tissue and fungal endophytes could alter the chemical environment, thus rendering it less attractive for *A. colombica*.

Additionally, the leaf could be less attractive due to chemicals produced by the fungal endophytes that are capable of producing several novel secondary metabolites (Sumarah and Miller, 2009). The foliar-fungal endophyte *Phialocephala scopiformis* (Helotiales: Mollisiaceae) produces the anti-insect compound 'rugulosin', when residing in some species of Pinaceae, thus negatively affecting the growth and development of *Choristoneura fumiferana* (Lepidoptera: Tortricidae) (Sumarah and Miller, 2009). Fungi generally known to live as either heterotrophs (e.g., species of *Alternaria*, Pleosporales: Pleosporaceae) or latent pathogens (several species of the Botryosphaeriaceae) or entomopathogenic (*B. bassiana*) exist as endophytes in leaves of plants. Established entomopathogenic fungi such as species of *Beauveria*, *Isaria* (Hypocreales: Cordycipitaceae), and *Metarhizium* (Hypocreales: Clavicipitaceae) survive as endophytes in tissues of both non-crop and crop plants (Vega, 2008). Vega et al. (2008) demonstrated that the two entomopathogenic fungi, *viz*., *B. bassiana* and *Clonostachys rosea* (Hypocreales: Bionectriaceae) living as endophytes in laboratory-maintained *Coffea arabica* (Rubiaceae) are potentially capable of regulating populations of *Hypothenemus hampei* (Coleoptera: Curculionidae). The ability of such entomopathogenic fungi to colonize and survive in plants as endophytes and to protect their host plants against insects has enhanced the hope of using endophytes for biological management of pestiferous arthropods and heterotrophic fungi such as *Botrytis cinerea* (Helotiales: Sclerotiniaceae) (Bamisile et al., 2018; Mantzoukas and Eliopoulos, 2020).

However, more studies are necessary to understand several aspects including the host preference and density of colonization of different crop plants by

entomopathogenous endophytes, and their interaction with other endophytes (Jaber and Ownley, 2018) before employing them as useful biological-management agents. It is further important to note that the ambient environment, soil nutrients, agricultural practices, and soil-microbial structure influence the establishment of the endosymbiotic community of a plant (Mighell et al., 2019). For instance, Suryanarayanan et al. (2018) showed that entomopathogenic fungal genera rarely colonize leaves of forest trees as foliar endophytes. It is also essential to screen non-entomogenous endophytes for their potential to ward of plant pests. *Trichoderma harzianum* (Hypocreales: Hypocreaceae) endophytic in *Sargassum wightii* (Phaeophyceae: Fucales: Sargassaceae) produced metabolites, which reduced the feeding on the leaves by the caterpillars of *Helicoverpa armigera* (Lepidoptera: Noctuidae); plants inoculated with *T. harzianum* had significantly fewer eggs of *H. armigera* (Suryanarayanan et al., 2018). *Trichoderma harzianum*, however, could not survive as an endophyte when sprayed as spores in crop plants for more than three weeks indicating that colonization of a plant by an alien endophyte may be viable only for 2–3 weeks (Suryanarayanan et al., 2018). Thus, even if an endophyte species with insect deterring properties is identified, methods to prolong its presence in a non-host crop have to be addressed for increasing its efficiency as a biological-management agent. Notwithstanding the inhibitory effects of fungal endophytes, plant-feeding insects could aid in the dispersal of endophytic fungal inoculum in the plant community. *Poekilocerus pictus* (Orthoptera: Pyrgomorphidae) when fed with the leaves of *Calotropis gigantea* (Apocynaceae) leaves, the leaf endophytes pass through their guts without being digested, retain their viability, could grow and sporulate on fecal pellets (Devarajan and Suryanarayanan 2006). This suggests that phytophagous insects could function as agents for the dispersal of fungal endophytes in plant communities such as tropical forests.

CONCLUSION

Understating more comprehensively the interactions among three participants belonging to three different kingdoms *viz*. fungal endophytes, plants and insects, constituting one complex, intricately intertwined system would strengthen efforts to managing plant loss by pestiferous insects with lesser damage to the environment.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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